▶**AAAP Symposium**
Current Respiratory Challenges in Poultry
A Perspective from the Field

JULY 29 - AUGUST 2 2022

AAAP

Symposium & Scientific Program

THE AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS

Facilitates member collaboration to advance science-based knowledge, expertise, and education on poultry health, welfare, and food safety.

Philadelphia, PA
July 29 - August 2, 2022

AAAP Event Schedule

Saturday

Scientific Symposium
11:30 AM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center

Opening Session
4:15 PM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center

Keynote Address
Douglas Fulnechek
4:30 PM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center

Welcome Reception and Poster Session
5:15 PM: Broad Street Atrium
Philadelphia Convention Center

Sunday

New Member Meet & Greet
5:30 PM: Philadelphia Ballroom South
Sheraton Philadelphia Downtown Hotel

Women's Network Dinner
6:30 PM: Horizons Rooftop Ballroom
Sheraton Philadelphia Downtown Hotel

Monday

Yoga
6:30 AM: Horizons Rooftop Ballroom
Sheraton Philadelphia Downtown Hotel

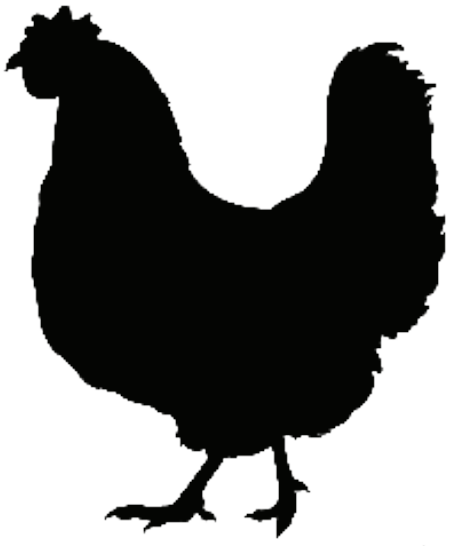
History Lecture
Jim McKay
10:15 AM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center

Business Meeting
10:45 AM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center

Awards Reception & Dinner
5:30 PM: Liberty Ballroom Foyer
6:00 PM: Liberty Ballroom
Sheraton Philadelphia Downtown Hotel

Tuesday

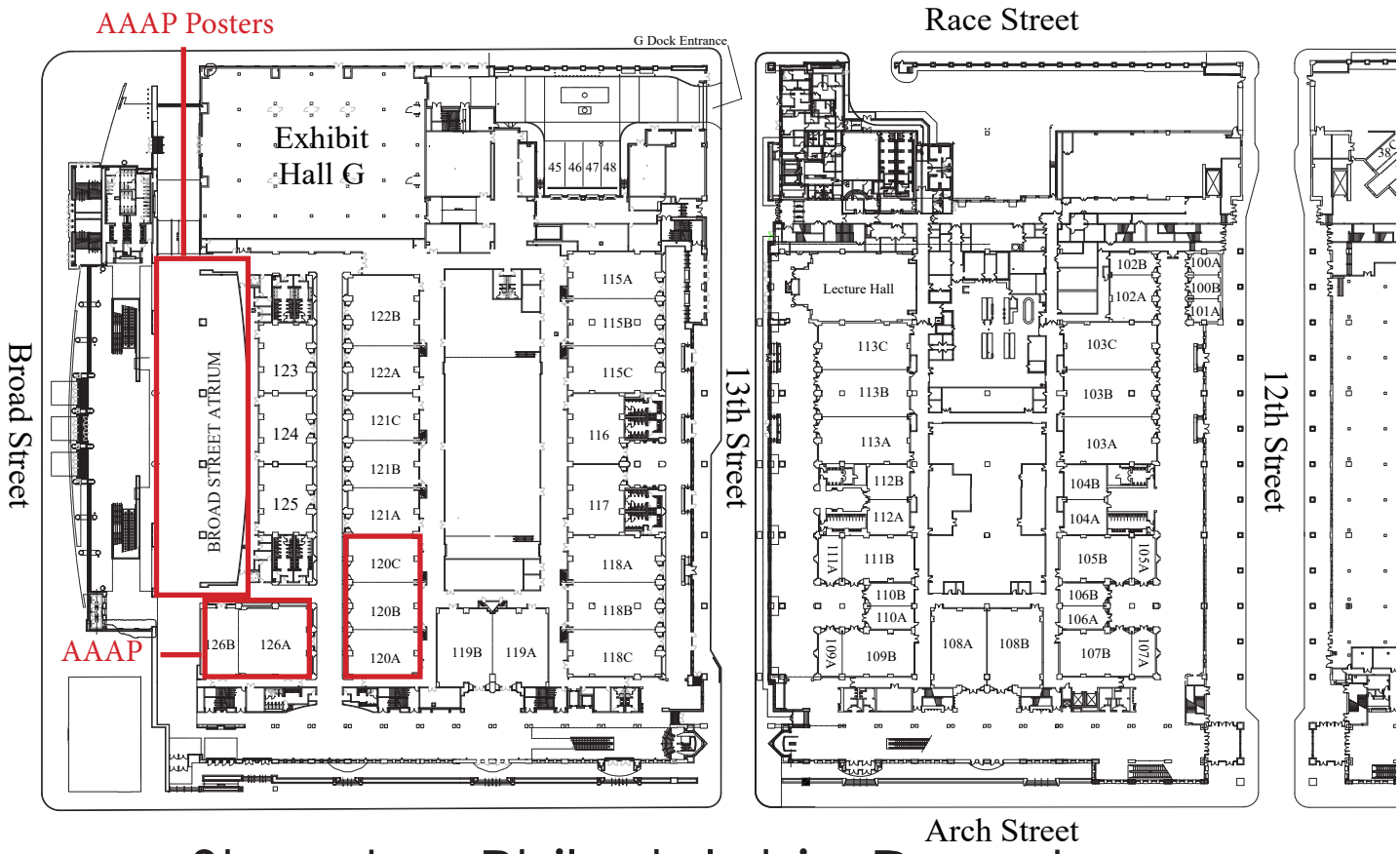
Wellness Talk
11:00 AM: Philadelphia Convention Center 126 AB
Philadelphia Convention Center



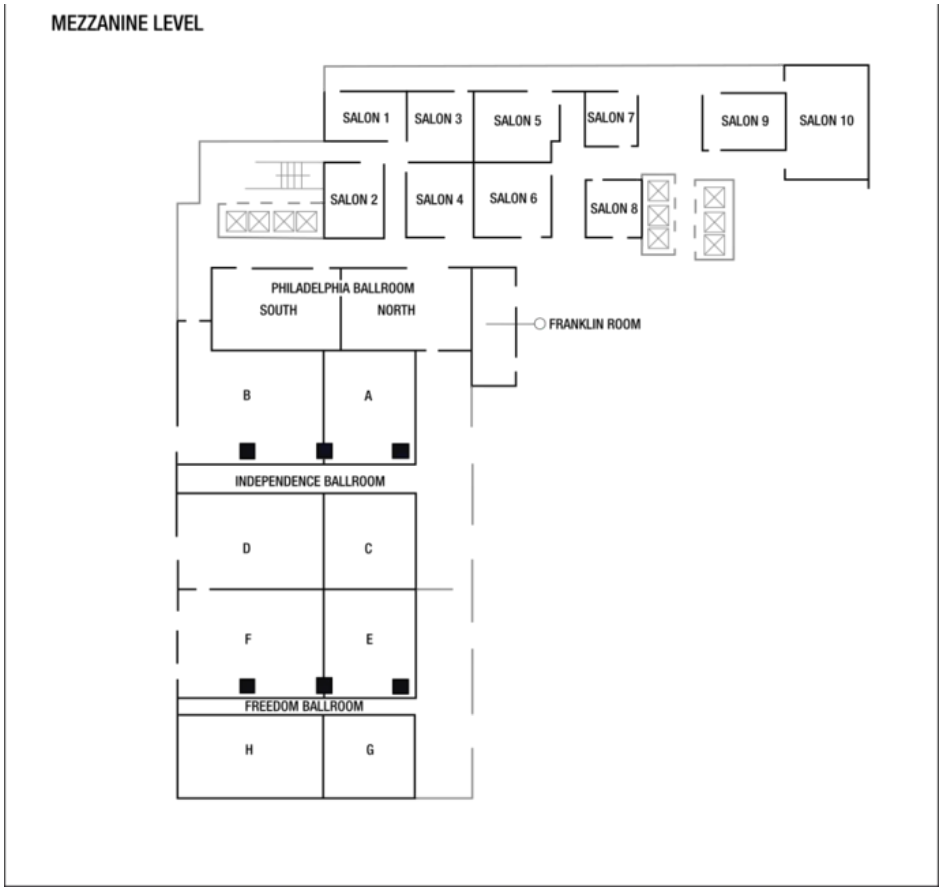
Name of Group	Meeting Date	Beg. Time	End Time	Location	Room
AAAP Board of Directors Meetings					
AAAP Inc. and Foundation Board of Directors	Friday, July 29	7:00 AM	5:00 PM	Sheraton	Parlor B
AAAP Past Presidents Luncheon	Monday, August 1	12:00 PM	1:30 PM	Sheraton	Salon 5-6
AAAP Committee Chairs and BOD Meeting	Tuesday, August 2	1:00 PM	5:30 PM	Sheraton	Salon 10
Committee Meetings					
Avian Diseases Editorial Board	Tuesday, July 12	9:00 AM	11:00 AM	Virtual	Virtual Meeting
Avian Diseases Advisory Board	Tuesday, July 12	11:00 AM	12:00 PM	Virtual	Virtual Meeting
Avian Diseases Reviewers Board	Tuesday, July 12	12:00 PM	1:00 PM	Virtual	Virtual Meeting
Preceptorship Committee	Wednesday, July 13	11:00 AM	12:00 PM	Virtual	Virtual Meeting
Diseases of Public Health Significance	Wednesday, July 13	2:00 PM	3:00 PM	Sheraton	Virtual Meeting
History Committee	Monday, July 25	4:00 PM	5:00 PM	Virtual	Virtual Meeting
Small Flocks Committee	Saturday, July 30	8:00 AM	9:00 AM	Sheraton	Freedom Ballroom E
Respiratory Diseases Committee	Saturday, July 30	8:00 AM	9:00 AM	Sheraton	Independence Ballroom AB
Education Committee	Saturday, July 30	8:00 AM	9:00 AM	Sheraton	Freedom Ballroom F
AAAP Auditing Committee	Saturday, July 30	8:00 AM	9:00 AM	Sheraton	Salon 2
Avian Diseases Manual Editorial Board	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Salon 5
Diversity & Inclusion Committee Meeting	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Independence Ballroom C
Animal Welfare Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Philadelphia Ballroom North
AAAP Foundation Scholarship Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Independence Ballroom D
Awards Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Salon 6
LAC Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Salon 10
Outreach Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Salon 9
Membership Committee	Saturday, July 30	9:00 AM	10:00 AM	Sheraton	Philadelphia Ballroom South
Research Priorities Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Salon 10
Epidemiology Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Freedom Ballroom F
Tumor Virus Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Freedom Ballroom GH
Enteric Diseases Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Philadelphia Ballroom North
Toxic, Infectious, Miscellaneous & Emerging Diseases Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Independence Ballroom AB
Drugs and Antimicrobials Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Freedom Ballroom E
Food Safety Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Independence Ballroom D
AAAP Foundation Development Committee	Saturday, July 30	10:00 AM	11:00 AM	Sheraton	Independence Ballroom C

Program Events					
Scientific Program Poster Room	Friday, July 29	8:00 AM	5:00 PM	PCC	Broad Street Atrium
Histopathology/Case Report Interest Group	Friday, July 29	1:00 PM	5:00 PM	Marriott	Franklin Hall 13
Small Flocks Interest Group	Friday, July 29	1:00 PM	5:00 PM	Marriott	Franklin Hall 12
Mentor/Mentee Breakfast (Invitation Only)	Saturday, July 30	7:00 AM	8:00 AM	Sheraton	Horizons Rooftop Ballroom
AAAP Symposium	Saturday, July 30	11:30 AM	4:00 PM	PCC	126 AB
AAAP Opening Session	Saturday, July 30	4:15 PM	4:30 PM	PCC	126 AB
AAAP Scientific Program Keynote Address	Saturday, July 30	4:30 PM	5:15 PM	PCC	126 AB
AAAP Welcome Reception and Poster Session	Saturday, July 30	5:15 PM	6:30 PM	PCC	Broad Street Atrium
AAAP Diversity & Inclusion Dinner and Meeting	Saturday, July 30	6:30 PM	8:00 PM	Sheraton	Philadelphia Ballroom North
AAAP Committee Chair/BOD Lunch Meeting	Sunday, July 31	12:15 PM	12:45 PM	Sheraton	Independence Ballroom C
AAAP New Member Meet & Greet	Sunday, July 31	5:30 PM	6:30 PM	Sheraton	Philadelphia Ballroom South
AAAP Women's Network Committee Meeting	Sunday, July 31	6:30 PM	9:00 PM	Sheraton	Horizons Rooftop Ballroom
Yoga	Monday, August 1	6:30 AM	7:30 AM	Sheraton	Horizons Rooftop Ballroom
AAAP Lasher-Eckroade History Lecture	Monday, August 1	10:15 AM	10:45 AM	PCC	126 AB
AAAP Business Meeting	Monday, August 1	10:45 AM	12:00 PM	PCC	126 AB
AAAP Awards Reception before Dinner	Monday, August 1	5:30 PM	6:00 PM	Sheraton	Liberty Ballroom Foyer
AAAP Awards Banquet	Monday, August 1	6:00 PM	8:00 PM	Sheraton	Liberty Ballroom A
Wellness Talk	Tuesday, August 2	11:00 AM	12:00 PM	PCC	120ABC
ACPV					
ACPV Board of Governors Meeting	Monday, August 1	7:00 AM	10:00 AM	Sheraton	Freedom Ballroom E
ACPV Annual Meeting/Breakfast	Tuesday, August 2	7:00 AM	8:30 AM	Sheraton	Independence Ballroom AB
Invitation Only					
Association of Veterinarians in Broiler Production Meeting	Friday, July 29	8:00 AM	3:00 PM	Marriott	Grand Ballroom E
Association of Veterinarians in Turkey Production	Friday, July 29	8:00 AM	5:00 PM	Marriott	Grand Ballroom A
Association of Veterinarians in Egg Production	Friday, July 29	12:00 PM	5:00 PM	Marriott	Grand Ballroom F
Association of Poultry Primary Breeder Veterinarians	Saturday, July 30	11:30 AM	1:30 PM	Sheraton	Philadelphia Ballroom South
NC Veterinarians and NCSU Students and Alumni	Sunday, July 31	7:00 AM	8:00 AM	Sheraton	Independence Ballroom C
MAM and MAHM Alumni Association	Tuesday, August 2	5:30 PM	7:00 PM	Sheraton	Philadelphia Ballroom North

Convention Center



Sheraton Philadelphia Downtown



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Enteric Diseases
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Research Priorities
Joel Cline 2023

Tumor Virus
Algis Martinez 2024

Toxic, Infectious, Miscellaneous and Emerging Diseases
Elizabeth Beilke 2024

Small Flocks
Annika McKillop 2022

Women's Network
Holly Sellers 2022

Outreach
Bernard Beckman 2022

Committee Review
Bruce Stewart-Brown

2021-2022 AAAP Board of Directors

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Saturday, July 30, 2022

AAAP Symposium: Current Respiratory Challenges in Poultry…A Perspective from the Field

Room	**126AB**
11:30 AM	**Introduction and Welcome** Dr. Rodrigo Gallardo
Moderator	Dr. Alejandro Banda
11:35 AM	**Effects of the House of Environment Management on Respiratory Health** Dr. Guillermo Zavala, *Avian Health International, LLC*
12:05 PM	**New Developments on Mass Vaccine Application Methods** Dr. Brian Jordan, *University of Georgia*
Moderator	Dr. Mark Jackwood
12:35 PM	**Discussion Panel on Current Problems Associated with Infectious Bronchitis** Panelists: Dr. Phil Stayer, *Sanderson Farms* Dr. David French, *University of Georgia* Dr. Carl Heeder, *Mountaire Farms* Dr. Deirdre I. Johnson, *Perdue Farms* Dr. Charles Corsiglia, *Foster Farms* Dr. Tyler Gamble, *Pilgrims*
1:15 PM	Break
Moderator	Dr. Simone Stoute
1:30 PM	**Current Topics on Fowl Cholera** Dr. Mark Bland, *Cutler Associates*
2:10 PM	**New developments on infectious coryza** Dr. Rodrigo Gallardo, *University of California, Davis*
2:40 PM	**Major respiratory issues of turkeys** Dr. Eric Gonder, *Butterball*
Moderator	Dr. Maricarmen Garcia
3:10 PM	**Discussion Panel on current problems associated with infectious laryngotracheitis** Panelists: Dr. Louise Dufour-Zavala, *Georgia Poultry Lab* Dr. Mark Burleson, *Wayne Farms* Dr. Donald Ritter, *Poultry Business Solutions LLC* Dr. Nancy Reimers, *Cutler Associates*
3:50 PM	**Final Announcements** Dr. Rodrigo Gallardo
4:00 PM	**Adjourn**

Scientific Program Begins

Room	**126AB**
4:15 PM	**Opening Session**
4:30 PM	**Keynote Speaker:** Douglas Fulnechek, *Zoetis* **Poultry, Salmonella, Campylobacter, and Food Poisoning**
5:15 PM	**Welcome Reception & Poster Session**

Sunday, July 31, 2022

Room	126AB		120ABC
Topic	**Avian Influenza**		**Enteric Health**
Moderator	Christina Leyson		John Schleifer
8:00 AM	**<u>Session Keynote:</u>** **Global Evolution and Intercontinental Spread of H5Nx clade 2344b High Pathogenicity Avian Influenza Viruses since 2020** David Swayne, *US Department of Agriculture*		**Comparison of Antimicrobial Susceptibility Testing of Clostridium perfringens by the Agar Dilution and the Broth Microdilution Methods** Martine Boulianne, *Université de Montréal*
8:15 AM			**Natural Prevention of Coccidiosis and Necrotic Enteritis in Experimentally Infected Broilers and Analysis of Gene Expression in Intestinal Tissue Samples** Felipe Mendy, *BIOVET S.A.*
8:30 AM	**Evaluation of Licensed and In-House Vaccines Against Recent H5 Low Pathogenicity Avian Influenza viruses** Miria Criado, *University of Georgia*		**Effect of A Feed Sanitizer on The Control of Necrotic Enteritis in Broilers** Enrique Montiel, *Anitox Corporation*
8:45 AM	**Effect of co-infection of low pathogenic avian influenza H9N2 virus and Avian pathogenic E. coli on H9N2 vaccinated commercial broiler chickens.** Wael Elfeil, *Suez Canal University*		**Experiences using a Recombinant Attenuated Salmonella Vaccine to control Necrotic Enteritis in Commercial Broilers** Andrea Zedek, *Huvepharma*
9:00 AM	**Development and Characterization of a Non-transmissible H5N2 Live Attenuated Avian Influenza Virus Vaccine Based on Altered Viral M2/M42 Ion Channel Expression That Protects Against Highly Pathogenic Virus Challenge** Darrell Kapczynski, *USDA-ARS-Southeast Poultry Research Laboratory*		**Beneficial effects of tea extract and cinnamon oil on avian coccidiosis** Inkyung Park, *USDA-ARS*
9:15 AM	**Phylogenetic analysis of H5N1 clade 2.3.4.4b HPAI viruses in wild birds and poultry in US in 2022** Sungsu Youk, *U.S. National Poultry Research Center, ARS-USDA*		**The impact of Salmonella Typhimurium and coccidiosis vaccine on microbiome and intestinal integrity markers in broiler chickens** Andrea Pietruska, *Auburn University*
9:30 AM	**Investigation of a Low Path Influenza Outbreak on a Turkey Breeder Farm** Marion Garcia, *Hybrid Turkeys*		**Differential Analysis of Jejunal mRNA of Broiler Chickens Challenged with Eimeria maxima with or without Clostridium perfringens** Nima Emami, *University of Georgia*
9:45 AM	**Dust as a Vehicle and Surveillance Sample of H5N8 Highly Pathogenic Avian Influenza Virus (Clade 2.3.4.4b) in Poultry Farms, France, 2021** Jean-Luc Guerin, *ENVT, University of Toulouse*		**Identification of arthropod vectors of Histomonas meleagridis and Heterakis gallinarum in broiler breeder farms** Maria Tereza Bethonico Terra, *Auburn University*
10:00 AM	**Break**		
Topic	**Avian Influenza**		**Enteric Health**
Moderator	Darrell Kapczynski		Enrique Montiel
10:15 AM	**Temperature and pH stability of low and high pathogenicity avian influenza viruses** Christina Leyson, *Southeast Poultry Research Laboratory*		**Focal Duodenal Necrosis: identification of Gram-negative rod-shaped bacteria in intestinal lesions** Yu-Yang Tsai, *The University of Georgia*

10:30 AM	**Addressing common obstacles to bird transfer movements during a highly pathogenic avian influenza outbreak – a cross-commodity approach** Rosemary Marusak, *University of Minnesota*		**An Update on Turkey Viral Enteritis Diagnostic Cases in Commercial Turkey Poults in California** Shayne Ramsubeik, *California Animal Health and Food Safety Laboratories Turlock, UC Davis*
10:45 AM	**Comparison of the pathogenicity in chickens and turkeys of a US 2022 wild bird H5N1 HPAI virus, clade 2.4.4.4b, and two previous clade 2.3.4.4 H5N8 HPAI viruses** Mary Pantin-Jackwood, *Southeast Poultry Research Laboratory*		**Surveillance and diagnosis of turkey coronavirus using environmental bootie swabs** Becky Tilley, *Butterball LLC*
Topic	**Immunology**		**Salmonella**
Moderator	Darrell Kapczynski		Enrique Montiel
11:00 AM	**Development of a High Interferon-Inducing Live Attenuated Influenza Vaccine for In Ovo Vaccination of Chickens** Amir Ghorbani, *The Ohio State University*		**Understanding NPIP's Pullorum-Typhoid Clean Programs** Elena Behnke, *USDA National Poultry Improvement Plan*
11:15 AM	**Systemic and mucosal lymphoid immune responses during Clostridium perfringens-induced necrotic enteritis in broiler chickens** Ravi Kulkarni, *North Carolina State University*		**Genetic characterization of Salmonella Infantis recovered from comminuted turkey samples collected by the USDA Food Safety and Inspection Service between 2019 and 2021** Roxana Sanchez-Ingunza, *RSI Poultry Veterinary Consulting LLC*
11:30 AM	**Characterizing Pro- and Anti-viral Mirna Regulated Pathways for Newcastle Disease Virus Replication** Abhijeet Bakre, *USDA*		**Impact of a New Vaccination Approach in Pullets and Broiler Breeder Layers to Reduce Salmonella Contamination in Broilers.** Jaime Ruiz, *Elanco Animal Health*
11:45 AM	**How Many Antigens Can You Give a Pullet: A Field Study Assessing How Multiple Vaccines Influence Antibody Titers** Gunnar Dunnam, *Mississippi State University*		**Comparison of Whole Cell Bacteria and SRP Salmonella Vaccines in Broiler Chickens: Evaluation of Protection Against Homologous and Heterologous Salmonella Strains** G Ritter, *Poultry Business Solutions LLC*
12:00 PM	**Lunch Break**		
Room	**126AB**		
Topic	**HPAI Focus**		
Moderator	Ivan Alvarado		
1:00 PM	**Vaccine Options for Highly Pathogenic Avian Influenza in the United States** David Suarez, *USDA*		
Room	**126AB**		**120ABC**
Topic	**Vaccinology**		**Case Reports**
Moderator	Ivan Alvarado		Yuko Sato
1:15 PM	**Pasteurella multocida Vaccines with Lower or No Post Vaccination Reaction Adjuvants in a Fowl Cholera Challenge Model** Charles Hofacre, *Southern Poultry Research Group, Inc.*		**Outbreak Of Multiple Subtypes Of Low-Pathogenic Avian Influenza In Waterfowl And Gamebird Flocks** Carmen Jerry, *UC Davis*
1:30 PM	**Experiences with a safe live Pasteurella multocida vaccine** Chris Morrow, *Bioproperties*		**Case Report: Investigating Unusual Outbreaks of Neurologic Disease in Broiler Chickens** Natalie Armour, *Mississippi State University*
1:45 PM	**Vaccination Trials in a Midwestern Heavy Tom Complex Using a Clostridium septicum Bacterin/Toxoid** Andrew Smith, *Butterball*		**My Birds Seemed Terrified!** Martha Pulido Landinez, *Mississippi State University*

2:00 PM	**Development of a Novel Vaccine Strategy Against Necrotic Enteritis by Synergizing Immune-enrichment and Immunostimulation in Broiler Chicks** Hemlata Gautam, *University of Saskatchewan, Canada*		**Cleaning up an Outbreak** Laura Tensa, *Cargill*
2:15 PM	**Effect of Coccidial Vaccines on the Stability of Salmonella and Viral Live Attenuated Vaccines** Alejandro Banda, *Mississippi State University*		**Histomonas meleagridis outbreak & transmission pathway investigation on a private farm in Tennessee, United States** Richard Gerhold, *University of Tennessee College of Veterinary Medicine*
2:30 PM	**The Case of the Missing Intestines: Peck-Outs in Broiler Breeders** Christina Lindsey, *Aviagen*		**A Pestilential Poult Problem** Jacob Carlson, *Select Genetics*
2:45 PM	**Protection of Ultifend® & Rispens vaccine against Infectious Bursal Disease Virus Variant E Challenge** Olivia Faulkner, *Ceva*		**Case report: Clinical investigation of a turkey barn with a recurrent history of histomoniasis** Vijay Durairaj, *Huvepharma Inc*
3:00 PM	**Break**		
Room	**126AB**		**120ABC**
Topic	**Reovirus**		**Case Reports**
Moderator	Richard Fulton		Jarra Jagne
3:15 PM	**Genetic and Antigenic Relatedness of Avian Reovirus Variants** Sofia Egana, *UC Davis*		**Increased Cull Eggs in an Upper Midwest Turkey Breeder Operation** Benjamin Wileman, *Select Genetics*
3:30 PM	**Antigenic Variation and In Vitro Neutralization of Avian Reovirus Isolates from Mississippi** Rebecca Mackey, *Poultry Research and Diagnostic Lab, Mississippi State University*		**Investigation into an Outbreak of Turkey Coronavirus and Histomonas Infections on Several Turkey Farms** Brian Wooming, *Cargill Protein*
3:45 PM	**Genetic, Antigenic, and Pathotypic characterization of newly emerging Turkey Hepatitis Reovirus** Sunil Mor, *University of Minnesota*		**Investigating urolithiasis in broiler breeders** Randi Clark, *Sanderson Farms*
Topic	**Diagnostics**		**Case Reports**
Moderator	Richard Fulton		Jarra Jagne
4:00 PM	**<u>Session Keynote</u>:** **Get the flu out of here…HPAIV environmental stability in outbreak waste and environmental testing strategies** Erica Spackman, *SEPRL-USDA-ARS*		**Broiler breeder pullets: wrong diet, high mortality, unusual lesions!** Tahseen Abdul-Aziz, *Rollins Animal Disease Diagnostic Laboratory*
4:15 PM			**Case Report: Kinda Wish it was the M Word!** Sara Throne, *Simmons Foods Inc*
4:30 PM	**Nanopore Sequencing: A Promising Tool for Fast Identification and Accurate Characterization of IBV Directly From Clinical Samples** Brittany Skaggs, *Iowa State University College of Veterinary Medicine*		**Case Report: HPAI in a Maryland Broiler Farm** Michael Quist, *University of Georgia, PDRC*
4:45 PM	**Fast Identification of Emerging Avian Viruses Using Nanopore Sequencing Device** Guillaume Croville, *ENVT - University of Toulouse*		**What Are Your Chickens Drinking?** Kurt Dobson, *George's Inc.*

Monday, August 1, 2022

Room	126AB		120ABC
Topic	**Diagnostics**		**Case Reports**
Moderator	Christina Lindsey		Carrie Cremers
8:00 AM	**This is How Today's Commercial Meat type and Egg Type Chickens Became ALV-Free** Guillermo Zavala, *Avian Health International, LLC*		**Case Report of Lessons Learned with Mycoplasma Synoviae Surveillance** Philip Stayer, *Sanderson Farms*
8:15 AM	**Biomarkers for Rapid Identification of White Chick Syndrome (Astrovirus) Cases at the Hatchery** Daniel Venne, *Couvoir Scott ltée*		**Epidemiologic Considerations from an HPAI outbreak in Dubois County Indiana** Duane Murphy, *Farbest Farms*
8:30 AM	**Infectious bronchitis virus and chicken laryngotracheitis infectious virus in backyard poultry located around breeder poultry farms in Brazil** Helena Lage Ferreira, *University of Sao Paulo*		**A peculiar Avibacterium paragallinarum Infection in Layers with Complete Absence of any Clinical Presentation of Infectious Coryza** Amro Hashish, *Iowa State University*
Topic	**Epidemiology**		**Pathology**
Moderator	Christina Lindsey		Carrie Cremers
8:45 AM	**Epidemiological investigation of MS in broiler breeders using the 14 NPIP biosecurity principles** Louise Dufour-Zavala, *GPLN*		**West Nile Virus Infection in a Developer Pekin Duck Flock** Richard Fulton, *Michigan State University*
9:00 AM	**Development of a multilocus sequence typing scheme for Avibacterium paragallinarum** Mostafa Ghanem, *University of Maryland-College Park*		**Reoccurrence of West Nile Virus Infection in Pekin Breeder Ducks Associated with a Drop in Egg Production** Mayra Tsoi, *Michigan State University*
9:15 AM	**A time-space investigation for better understanding the epidemiology of Ornithobacterium rhinotracheale in commercial turkeys in Iowa** Yuko Sato, *Iowa State University*		**An unusual case of systemic Histomoniasis in a backyard turkey poult** Emily Pittman, *Georgia Poultry Laboratory Network*
9:30 AM	**Multistate Psittacosis Outbreak at Chicken Slaughter Plants— Virginia and Georgia, 2018** Christine Szablewski, *CDC*		**Black Livers at Processing in Broiler Chickens** Veronica Nguyen, *Department of Population Health and Reproduction, UC Davis School of Veterinary Medicine*
9:45 AM	**Turkey Coronavirus Enteritis: Lessons and Experiences From the Field** Eric Orozco, *Butterball*		**Spontaneous Testicular Teratoma and Facial Xanthomas in a Spitzhauben Rooster** Jarra Jagne, *Cornell University Animal Health Diagnostic Center*
10:00 AM	**Break**		
Room	**126AB**		
10:15 AM	**History Lecture:** The History of Poultry Breeding Jim McKay, EW Group		
10:45 AM	**Business Meeting**		
12:00 PM	**Lunch**		
Room	**126AB**		
Topic	**HPAI Focus**		
Moderator	Alejandro Banda		
1:00 PM	**Epidemiology of the 2022 H5N1 HPAI outbreak in the U.S.** Julie Gauthier, *USDA*		

Room	126AB		120ABC
Topic	**Infectious Bronchitis Virus**		**Coccidiosis**
Moderator	Alejandro Banda		Jaime Ruiz
1:15 PM 1:30 PM	**<u>Session Keynote</u>:** **IBV surveillance as a tool in a disease prevention program** Rodrigo Gallardo, *University of California, Davis*		**<u>Session Keynote</u>:** **Using Eimeria Lesion Scores and Fecal Oocyst Counts in Addition to Final Body Weight as Parameters in Floor Pen Studies** Rüediger Hauck**,** *Auburn University*
1:45 PM	**Assessment of infectious bronchitis virus (IBV) exposure risks in broiler chicken using spatial analysis, machine learning, and risk factor analysis.** Andreia Arruda, *The Ohio State University*		**OPGs: What do they mean?** Hector Cervantes, *The University of Georgia*
2:00 PM	**Proactive management of Infectious Bronchitis Virus (IBV) challenge in commercial broilers: Lessons learned from field cases** Matilde Alfonso, *Ceva Animal Health*		**Validation a Portable Cell Counter for Enumeration of Eimeria species of Chickens** Rocio Crespo, *North Carolina State University*
2:15 PM	**Comparison of Infectious Bronchitis Virus Surveillance Profiles in Layers, Broiler Breeders, and Broilers** Brian Jordan, *The University of Georgia*		**Evaluation of Litter Moisture Analyzers and the Effects of Litter Moisture on Cycling of Coccidiosis Vaccine** Nicholas Brown, *Huvepharma Inc*
2:30 PM	**The Costs of Controlling DMV/1639: A Comparison of Vaccination Strategies and Their Effects on Broiler Livability, Vaccine Clearance, and Performance** Blayne Mozisek, *Merck Animal Health*		**Analyzing Nanopore NGS Data of Vaccine and Field Isolate Eimeria for Identification** Benjamin Jackwood, *UGA*
2:45 PM	**Evaluating the Effects of IBV Maternal Antibodies on the Development of False Layer Syndrome** Adrea Mueller, *University of Georgia*		**In Ovo Vaccination of Chickens Against Eimeria Maxima Infection Using Recombinant EmaxIMP1 Protein Linked to Nanoparticles** Mark Jenkins, *ARS-USDA*
3:00 PM	**Break**		
Topic	**Infectious Bronchitis Virus**		**Coccidiosis**
Moderator	Brian Jordan		Don Ritter
3:15 PM	**Vaccine Interaction and Protection Elicited by IBV Maternal Antibodies Against Early Challenge with DMV/1639** Rachel Jude, *University of California, Davis*		**Control of Poultry Coccidiosis by Vaccination in Pigmented Broilers in Mexico** Francisco Rios-Cambre, *MSD Salud Animal Mexico*
3:30 PM	**Evaluation of antibody response and virus shedding following Infectious Bronchitis Virus (IBV) vaccination with Ma5 and DMV/1639 in SPF layers** Roel Becerra, *University of Georgia Poultry Diagnostic & Research Center*		**Everything Comes Down to Poo - Most Effective Form of Vaccination Application Using a Commercially Available Live Coccidia Vaccine** Jolene Tourville, *Jennie-O Turkey Store*
3:45 PM	**Infectious Bronchitis ELISA antibody titers as an indicator of productive impact in naturally infected broiler flocks** Jorge Chacon, *Ceva Animal Health*		**Comparison of Broiler Performance Between Various Coccidia Vaccination Programs Using a Novel Rapid Oocyst-Per-Gram Enumeration Method** Andrew Bishop, *Amick Farms*
4:00 PM	**Tracing Genomic Mutations in Spike Proteins of Infectious Bronchitis Virus (IBV) for Predictions of Neutralizing Epitopes and/or Vaccine Development** Kim Bouwman, *University of Georgia*		**Effect of a Blend of Essential Oils on Growth Performance and Intestinal Lesions during a mixed Eimeria Challenge or Vaccination in Two Studies** Sharon Heins Miller, *Devenish Nutrition LLC*

4:15 PM	**Assessment of mixing coccidia and infectious bronchitis live vaccines for day of age application in chickens** Robert Beckstead, *Ceva Animal Health*		**The Impact of Coccidiosis Control Programs on the Field Performance of US Poultry Farms** Ha-Jung Roh, *Merck Animal Health*
Topic	**Wealth of Knowledge**		**Coccidiosis**
Moderator	Brian Jordan		Don Ritter
4:30 PM	**Changes to the 2021 OIE Chapter on Avian Influenza and the Economic and Trade Impacts of High Pathogenicity Avian Influenza on the U.S. Poultry Industry** Fidelis Hegngi, *USDA, APHIS*		**Production of chicken tumor necrosis factor-a (TNF-a) in coccidiosis and necrotic enteritis** Hyun Lillehoj, *USDA*
4:45 PM	**Quantifying Antimicrobial Use in Poultry Production** Randall Singer, *University of Minnesota*		**Changes in gut microbiome, immunity, antioxidant capacity and parasite fecundity induced by Oraltreatment of transgenic Bacillus subtilis-cNK2 in E. acervulina-infected broiler chickens** Samiru Wickramasuriya, *USDA-Agricultural Research Service*

Tuesday, August 2, 2022

Room	126AB		120ABC
Topic	**Wealth of Knowledge**		**Infectious Bursal Disease**
Moderator	Rosemary Marusak		Kalen Cookson
8:00 AM	**Report of the Research Priorities Committee 2022 survey results** Joel Cline, *Wayne Farms LLC*		**<u>Session Keynote</u>:** **Isolation and Characterization of Antigenic Variant Infectious Bursal Disease Viruses in the United States** Milos Markis, *AviServe LLC*
8:15 AM	**Does this "Stuff" Work? An Applied Poultry Researcher's Perspective on Experimental Design** Matthew Jones, *Southern Poultry Research Group, Inc.*		
8:30 AM	**Investigating true fertility of 'clear' eggs at transfer** Isabella Hannay, *University of Georgia PDRC*		**Evaluating The Antigenic Relatedness Of Diverse Infectious Bursal Disease Virus Strains** Vishwanatha Reddy Avalakuppa Papi Reddy, *The Pirbright Institute*
8:45 AM	**Electrostatic Application of a Bound Residual Antimicrobial to Broiler Breeder Eggs: Impact on Hatchability, Chick Quality and 7-day Mortality** Mary Thompson, *UGA Poultry Diagnostic & Research Center*		**Assessment of a Variant IBDV Challenge in Chickens Utilizing Combinations of Recombinant HVT and Live Attenuated IBD Vaccines** John ElAttrache, *Ceva Animal Health*
9:00 AM	**Controlled Exposure utilizing a Hemolytic Ornithobacterium rhinotracheale Strain in Commercial Hen Turkeys to Protect Against a Field Challenge** Jessica Walters, *Virginia Department of Agriculture and Consumer Services*		**Broiler Protection and Performance Trials of the Newest HVT-IBD Recombinant Vaccine Against AL2 and Group-6 IBDV Challenges** Kalen Cookson, *Zoetis*
9:15 AM	**First Week Broiler Mortality: Trends, Lesions, Etiologies and Recommendations** Jose Linares, *Ceva Animal Health*		**Deciphering the Mode of Action of MB-1, a Live Hatchery Vaccine against Gumboro Disease** Yossi Wein, *The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*
9:30 AM	**Applications Used to Help Control and Eradicate Low Pathogenic Avian Influenza From Commercial Turkey Farms** Carrie Cremers, *Jennie-O Turkey Store*		**Flow Cytometric Analysis of T Regulatory Cells in the Bursa Of Fabricius Of Chickens Infected With IBDV** Salik Nazki, *The Pirbright Institue*
9:45 AM	**Outreach Efforts and Newcastle Vaccine Efficacy to Promote Gamefowl Wellness in Southern California** Alejandra Figueroa, *UC Davis School of Veterinary Medicine*		**Evaluation of Host Genetic Resistance to Infectious Bursal Disease Virus** Julia Blakey, *USDA- ARS USNPRC*
10:00 AM	**Break**		
Room	**126AB**		**120ABC**
Topic	**Welfare**		**ILT/Marek's**
Moderator	Eric Gonder		Rocio Crespo
10:15 AM	**<u>Session Keynote</u>:** **Welfare Frameworks: History, Evolution and Practical Implementation** Katherine Weathers, *Cobb-Vantress, Inc*		**<u>Session Keynote</u>:** **Comparison of Marek's Disease Virus Challenge Strains in Commercial Broiler-type Chickens** John Dunn, *USDA-ARS-USNPRC*
10:30 AM			

10:45 AM	**The Barnyard Perspective of Animal Welfare: Learning from each other** Angela Baysinger, *Merck Animal Health*		**In ovo vaccination with HVT accelerates immunocompetence in chickens** Allison Boone, *North Carolina State University, College of Veterinary Medicine and North Carolina Veterinary Diagno*
11:00 AM	**Wellness Talk:** **Options for Loan Forgivness** Megan Lighty, *Penn State University* Annika McKillop, *USDA APHIS* Dan Wilson, *Wilson Veterinary Co.* Kevin Maschek, *Pilgrim's Pride* Alex Strauch, *Alexander Strauch Consulting* Michaela Olson, *Wilson Veterinary Co*		**Efficacy of a new trivalent vHVT-IBD-ILT vaccine administered to day-old pullets against virulent ILTV challenge performed at two time points** Andrea Delvecchio, *Boehringer Ingelheim AH*
11:15 AM			**HVT-ILT Recombinant Vaccines: Dynamics of Replication and Protection in Commercial Broilers** Ivan Alvarado, *Merck Animal Health*
11:30 AM			
12:00 PM	**Lunch Break**		
Room	**126AB**		
Topic	**HPAI Focus**		
Moderator	Nancy Reimers		
1:00 PM	**Update on HPAI in the US** Mia Torchetti, *National Veterinary Services Laboratories*		
Room	**126AB**		**120ABC**
Topic	**Welfare**		**ILT/Marek's**
Moderator	Nancy Reimers		Mohamed El-Gazzar
1:15 PM	**Cage Free Housing for Laying Hens: Floor Substrate Provided During Rearing Influences Welfare Parameters** Marisa Erasmus, *Purdue University*		**Comparing ILT Live Vaccine Efficacy Through Drinking Water and Eye Drop by On-Site PCR Test** Keat Fu, *Aviagen Inc.*
1:30 PM	**Applying the Assessment of Key Indicators in Layer Chicken Systems for Improved Welfare** Joe Sullivan, *Herbruck's*		**Lt Serology in Georgia Flocks in the Absence of a Broiler Outbreak** Len Chappell, *Georgia Poultry Laboratory Network*
1:45 PM	**Understanding welfare of laying hens in different types of cage-free housing systems** Darrin Karcher, *Purdue University*		**Expression of Interferons and Interferon-Stimulated Genes (ISGs) in Larynx, Trachea, and Conjunctiva of Chickens Ocularly Inoculated with Live Attenuated Vaccines and Virulent Strains of Infectious Laryngotracheitis Virus (ILTV)** Daniel Maekawa Maeda, *University of Georgia*
Topic	**Welfare**		**Bacteriology**
Moderator	Nancy Reimers		Mohamed El-Gazzar
2:00 PM 2:15 PM	**Emphasizing Welfare Outcomes and KWIs: NCC Broiler and Broiler Breeder Welfare Guidelines Update and Audit Changes** Ashley Peterson, *National Chicken Council*		**<u>Session Keynote</u>:** **Campylobacter hepaticus and its Role in the Emergence of Spotty liver disease in Layers** Silke Rautenschlein, *Clinic for Poultry, University of Veterinary Medicine, Hannover*
2:30 PM	**Broiler Chicken Welfare: Overview of Key Welfare Indicators Used in Broiler Production Systems and a Practical Approach to Achieve Continuous Improvement** Kathleen Long, *Maple Leaf Foods*		**Does a Post-biotic Product have a Protective Effect Against APEC Challenge?** Catherine Logue, *Univ of Georgia*
2:45 PM	**The Importance of Key Welfare Indicators in Driving Continuous Improvement in Animal Welfare Outcomes** Ken Opengart, *Tyson Foods*		**Enterococcus faecalis Modulates Virulence of Avian Pathogenic E. coli by Stimulating Growth and Production of Capsular Polysaccharides** Grayson Walker, *NC State University*
3:00 PM	**Break**		

Room	126AB		120ABC
Topic	**Welfare**		**Bacteriology**
Moderator	Kabel Robbins		Martine Boulianne
3:15 PM	**Effects of novel Pulsed Alternating Wavelength System (PAWS) on welfare and skeletal quality of laying hens** Brittney Emmert, *Purdue University*		**Identification of Novel Genes Involved in the Biofilm Formation Process of Avian Pathogenic Escherichia coli (APEC)** Meaghan Young, *University of Georgia*
3:30 PM	**Understanding Outcomes-Based Welfare Assessments and Internal Audits in Turkey Production Environments** Molly Parker, *Butterball, LLC*		**Early turkey mortality related to infection with Streptococcus gallolyticus** Ann Wooming, *Church and Dwight*
3:45 PM	**Potential Welfare Issues Affecting Turkey Husbandry and Well-Being** Helen Wojcinski, *Wojcinski Poultry Health Consulting*		**Genotypic Classification of Avibacterium paragallinarum, the Causative Agent of Infectious Coryza** Ana da Silva, *University of California, Davis*
4:00 PM	**Evaluation of Foot Pads throughout Production in Commercial Turkeys** Kabel Robbins, *Butterball, LLC*		**Development of Core Genome Multilocus Sequence Typing (cgMLST) Scheme for Genotyping of Pasteurella multocida** Mohamed El-Gazzar, *Iowa State University*
4:15 PM	**"Good as Hell": A Discussion Regarding Toe Treatments of Hen Poults** Katie Stumvoll, *Jennie-O Turkey Store*		**Case Report: Is The Prevalence of Clostridium Septicum Dermatitis and Septicemia Underestimated in Commercial Egg Layers?** Michaela Olson, *Wilson Veterinary Company*
4:30 PM	**A Comparison of Various Euthanasia Devices and Methods in Turkey Hens** Brian Wooming, *Cargill Turkey & Cooked Meats*		**Assessing Two Fertile Eggshell Interventions With Luminometry** Ricardo Munoz, *Neogen Corporation*
4:45 PM	**Welfare Communication Tools for Field Veterinarians** Nancy Reimers, *Cutler Associates*		**Stress during Rearing-Laying Transition changes Gut Microbiota Profile, Intestinal Cytokine Expression and Skeletal Properties in Commercial Laying Hens** Prafulla Regmi, *University of Georgia*
5:00 PM	**Adjourn**		

AAAP Posters

Antimicrobial/Antibiotic Resistance

1: Minimal inhibitory concentrations of avilamycin to Clostridium perfringens isolates from broiler chicken farms before and after the approval of Surmax® Premix (Avilamycin) in Canada
Eric Parent, *Elanco Canada*

2: The protective role of Coli-vac, a heptavalent vaccine against extraintestinal avian pathogenic Escherichia coli (APEC) infection
Wael Elfeil, *Suez Canal University*

3: Chicken Gut Microbiota Dynamics after Amoxicillin and Thiamphenicol Treatment
Andrea Laconi, *University of Padua*

Avian Influenza

4: Infection of H5N6 HPAIV and evaluation of inactivated H5N6 HPAI vaccine on ducks
Seojeong An, *Konkuk University of Veterinary Medicine*

5: Antigenic Characterization of Low Pathogenic Avian Influenza Virus H9N2 isolated in the Republic of Korea
Andrew Cho, *Konkuk University*

6: Evaluation protection of H5 vaccination regimes against early challenge with HPAI-H5N8 Clade 2.3.4.4
Wael Elfeil, *Suez Canal University*

7: Live Recombinant NDV-Vectored H5 Vaccine Protects Chickens and Domestic Ducks from Lethal Infection of the Highly Pathogenic H5N6 Avian Influenza Virus
Heesu Lee, *Konkuk University, Seoul*

8: Stepwise adaptation of Hemagglutinin of H5N2 Highly pathogenic avian influenza virus in chickens
Sungsu Youk, *USDA ARS US Natl Poultry Research Ctr*

Bacteriology

9: Molecular characterization of few Avibacterium paragallinarum isolates from different US states using whole genome sequencing
Mostafa Ghanem, *The Ohio State University*

10: Epidemiological Surveillance Of Chlamydophila Psittaci In Wild Birds From Perú
Rosa Gonzalez, *San Marcos University, Lima-peru*

11: Does the Nutritionist have a role to play in Managing Campylobacter jejuni in Broilers?
Matthew Jones, *Southern Poultry Research Group*

12: High Mortality Outbreaks in Broiler Chickens Caused by Highly Virulent and Multidrug Resistant Escherichia coli
Christopher Poulos, *Animal and Plant Health Agency*

Case Reports

13: Isolation and Characterization of Goose Tembusu Virus in Taiwan
Hui-Wen Chen, *National Taiwan University*

14: Imposter false layer syndrome
Emily Pittman, *Georgia Poultry Laboratory Network*

Coccidiosis

15: Sensitivity of Eimeria Field Isolates in the United States: An update on responses to current practices
Luis Gomez, *Phibro*

16: Alternatives Treatments for Coccidiosis and Necrotic Enteritis Management
Greg Mathis, *Southern Poultry Research, Inc.*

17: Intestinal Integrity (I2) Correlations to Chicken Gut Health
Francene Van Sambeek, *Elanco Animal Health*

Diagnostics

18: Utilization of the i-STAT1 handheld clinical analyzer and VetScan2 to compare blood values in normal vs. recumbent turkey hens at point of lay
Jewell Bremer, *Select Genetics*

19: Evaluation of Flongle and MinION Flow Cells for the Whole-Genome Sequencing of Avian Influenza A Virus in Clinical Samples
Iryna Goraichuk, *USDA/ARS/SEPRL*

20: FTA Card Use as a Sample Collection Method to Type Avibacterium paragallinarum
Rachel Jude, *University of California, Davis*

21: Analysis of diagnoses for broiler chicken flocks in Korea between 2017 and 2021
chung-hyun Kim, *QIA*

22: Identification of the causative agent in broiler chicken showing neurological symptoms
Hye-Ryoung Kim, *APQA*

23: Infectious bronchitis molecular surveillance in layer farms in Southern California
Evelin Saenz, *UCDAVIS*

Enteric Health

24: Feed quality assessment with the use of glucose oxidase vs antibiotics
Josue Sanchez, *excelling SA de CV*

25: Ileal mucosal microbiota in commercial turkey poults
John Ngunjiri, *Center for Food Animal Health, The Ohio State University*

26: Effects of different methionine to cysteine ratios on the gut health, oxidative status, gene expression of nutrient transporters, and tight junction proteins of broiler chickens challenged with Eimeria spp
G Liu, *Univeristy of Georgia*

ILT/Marek's Disease

27: Longitudinal Study of Infectious Laryngotracheitis Virus (ILTV) in Commercial Laying Farms from Sao Paulo State – Brazil
Renato Luciano, *Biological Institute - Advanced Center of Poultry Research*

28: Characterization of the Backyard (Non-Commercial, Non-Quota) Poultry Population in Alberta, Canada, and the Submission Level Prevalence of Infectious Laryngotracheitis Within this Sector
Heather Van Esch, *Government of Alberta, Ministry of Agriculture, Forestry and Rural Economic Development*

29: Evaluation of the impact of injector device on Marek's disease vaccine delivery by subcutaneous route
Andrea Delvecchio, *Boehringer Ingelheim AH*

30: Use of a Novel Medium to Grow Chicken Embryo Fibroblasts that Increases the Yield of Marek's Disease Vaccines
Isabel Gimeno, *North Carolina State University*

Immunology

31: Assessment of Seroconversion In Commercial Layers During The Rearing Period Using Three Different Elisa Kits After Administration Of An Immune-Complex Vaccine (Novamune®) At One-Day-Old.
Oscar Sanabria, *Ceva*

32: Comparison of Transcriptional Analysis, ELISA, and Biological Assays of Avian Type I IFN
Chang Lee, *Southeast Poultry Research Lab, USDA-ARS*

33: Immunometabolic regulation of CpG-ODN-induced antimicrobial trained immunity in chickens
Iresha Subhasinghe, *University Of Saskatchewan*

34: The impact of antibiotic growth promoters on intestinal health during a subclinical necrotic enteritis challenge in broilers
Shailes Bhattarai, *University of Georgia*

Infectious Bronchitis Virus

35: Phylogenetic Analysis of Infectious Bronchitis Virus Circulating in Colombia From 2015 to 2021
Oscar Sanabria, *Ceva*

36: Isolation, Genotyping and Evaluation of Pathogenicity of Different Egyptian Infectious Bronchitis Viruses
Manal Afify, *Faculty of Veterinary Medicine, King Salman International University*

37: Homologous and heterologous live-attenuated vaccines strategy to reduce infectious bronchitis virus transmission
Robert Beckstead, *Ceva Animal Health*

38: Isolation of Infectious Bronchitis virus variant strain from commercial layer birds showing cystic oviduct in India
Namdeo Bulbule, *Venkateshwara Hatcheries Pvt Ltd*

39: Real World Evidence: Economic Assessment of the Impact of Q1-like Infectious Bronchitis Variant in a Broiler Productive Zone in Peru
Claudia Carranza, *Ceva Animal Health*

40: Data Management as a Strategy for Serological Monitoring of Infectious Bronchitis in broiler farms
Jose Perez, *SPM, NCSU*

41: A broad protection spectrum against relevant strains can be achieved by the combination of Mass-type and BR-I-type infectious bronchitis vaccine strains
Jorge Chacon, *Ceva Animal Health*

42: Persistence and intensity of Infectious Bronchitis Virus Vaccine Strain replication and its association with safety aspects
Jorge Chacon, *Ceva Animal Health*

43: Complete Genome Analysis of Two Live Attenuated Infectious Bronchitis Virus Vaccine Candidates Provide Insight Into the Attenuation Mechanism
Yun Jeong Choi, *Konkuk University*

44: Genome Sequence Variations of Infectious Bronchitis Virus Serotypes from Commercial Broilers in Mexico
Henry Kariithi, *USDA-ARS*

45: Surveillance of Infectious bronchitis virus occurred in Korea, 2021
Hyun-jin Kim, *Avian Disease Laboratory, Konkuk University College of Veterinary Medicine*

46: Detection of GI-13 of infectious bronchitis virus in layer chickens in Brazil
Helena Lage Ferreira, *University of Sao Paulo*

47: Development of a live attenuated vaccine for Indonesian QX-like IBVs (GI-19) using heat-adapted attenuation platform
Hyuk-chae Lee, *KHAV*

48: Development of a Highly-Sensitive NGS Method for Analysis of the Complete S1 Region of the Spike Gene from Infectious Bronchitis Viruses Directly from Field Samples
Derek Moormeier, *Ceva Animal Health*

49: Detection of Novel Infectious Bronchitis Viruses in Layers in Mexico and their Control by Using a Synergistic Live Vaccine Combination
Francisco Rios-Cambre, *MSD Salud Animal Mexico*

Infectious Bursal Disease

50: Very virulent IBDV can efficiently replicate and be transmitted in layer-type naive chickens up to 16 weeks of age
Christophe Cazaban, *Ceva Animal Health*

51: Comparison of IBD Antibody ELISA Titers using Kits from Various Vendors
Brenda Glidewell, *Georgia Poultry Laboratory*

52: Developing a Novel Method for Sequencing and Genotyping of Infectious Bursal Disease Virus Directly from Field Samples
Julia McElreath, *Ceva Animal Health*

53: Presence and Distribution of Infectious Bursal Disease viruses belonging to Genotypes 2 and 5 in Mexico
Francisco Rios-Cambre, *MSD Salud Animal Mexico*

Mycoplasma

54: Ms-H Vaccine (Vaxsafe MS) Protection in Broiler Breeders.
Francisco Perozo, *Boehringer Ingelheim*

55: Monitoring of Mycoplasma gallisepticum live vaccine -K Strain –
Husam Al Bakri, *Vaxxinova International BV*

56: Serological monitoring of chicken flocks Vaccinated against Mycoplasma gallisepticum
Sung Il Kang, *Animal and Plant Quarantine Agency*

57: Why not within first week? Success cases of early vaccination with a live Mycoplasma gallisepticum vaccine in Asia
Ludio Martins Gomes, *Vaxxinova International*

Newcastle Disease Virus

58: Newcastle disease virus genotypes circulating in Africa and their determinants of virulence: a systematic review
Charlie Amoia, *Sokoine University of Agriculture / Southern African Centre for Infectious Disease Surveillance (SAC*

59: Evaluation of protection by ND vaccination protocols against early challenge with Velogenic Newcastle virus-VII.1 in commercial broiler with Maternal Immunity
Wael Elfeil, *Suez Canal University*

60: Epidemiological Surveillance of Avian Paramyxovirus-1 in Wild Birds from Perú
Eliana Icochea, *Universidad Nacional Mayor de San Marcos*

61: The Thermal Stability of Newcastle Disease Virus in Poultry Litter
Jongseo Mo, *Southeast Poultry Research Laboratory, US National Poultry Research Center, Athens, GA*

Parasitology

62: Toxoplasma gondii Prevalence in Waterfowl and Gulls From Eight States in the United States
Richard Gerhold, *University of Tennessee College of Veterinary Medicine*

63: Metabolic profile of Histomonas meleagridis and undefined bacterial population in Dwyer's media with and without rice starch
Richard Gerhold, *University of Tennessee*

Salmonella

64: Effect of AviPro™ Megan™ Vac 1 on growth performance in broiler chicks Oraly challenged with S. Thyphimurium wild strain
Priscilla Karina Koerich, *PRISCILLA KOERICH*

65: Efficacy evaluation of Salmonella Enteritidis SRP vaccine against challenge by Salmonella Enteritidis, S. Typhimurium and S. Gallinarum in brown laying hens
Gabrielle Bragaglia, *Vaxxinova*

66: Epidemiological investigation of S. typhimurium monophasic at a broiler integrator
Louise Dufour-Zavala, *GPLZN*

67: Quantitative Distribution and Interaction of Salmonella Zega with Host Cells in Visceral Organs of Chickens Infected through Three Routes
Fakilahyel Mshelbwala, *Federal University of Agriculture, Abeokuta*

68: Safety of two Salmonella vaccines and zootechnical performance after vaccination of laying hens for prevention of salmonellosis
Jeniffer PImenta, *Vaxxinova*

69: Isolation of Salmonella Spp from Trachea of Broiler Chickens and Analysis of its Potential Role as Monitoring Diagnostic Tool
Martha Pulido Landinez, *Mississippi State University*

70: Detection of Salmonella in Quail Eggs for Ambulatory sale in Districts of the City of Lima-Peru
Magali Salas, *ALFA BIOL S.A.C*

71: Changes in Salmonella Serotypes over the last 30-something years
Doug Waltman, *GPLZN*

Vaccinology

72: Duration of Ultifend® & Rispens protection against Newcastle disease virus
Olivia Faulkner, *Ceva*

73: Comparison of the Serologic Response of Two Vaccination Programs with Different IBV Antigen Fractions in Broiler Breeders
Antonio Cobian, *SPM, NCSU*

74: Evaluating IBV and NDV Monovalent or Bi-valent Vaccinations Take by On-Site PCR Test
Keat Fu, *Aviagen Inc.*

75: Development of an indirect Enzyme-Linked Immunosorbent Assay based on ILT gD recombinant protein for the monitoring of HVT-gD vaccine.
Marina Gaimard, *Innovative Diagnostics*

76: Compatibility of A Recombinant HVT-IBD Vaccine with CVI to Provide Protection Against Very Virulent Marek's and Very Virulent IBDV Challenge in SPF Birds
Angela Hartman, *Zoetis*

77: Construction of DIVA capable, low cytotoxic-endotoxic, and immune-competent attenuated Salmonella Gallinarium vaccine candidate
John Hwa Lee, *Jeonbuk National University*

78: New technology vaccines return on investment in commercial broilers under high challenge field condition
Hazem Negm, *Ceva Animal Health*

79: Determining Minimum Protective Dose of Inactivated Trivalent Fowl Adenovirus (4, 8b, 11) Vaccine
Dam-Hee Park, *Choong Ang Vaccine Laboratories*

Virology

80: Molecular characterization of circulating avian reovirus in Egypt
Ayman El-Deeb, *Evapharma*

81: Pathological investigation on Fowl Adeno virus infection in Middle East Area 2019-2021
Husam Al Bakri, *Vaxxinova International BV*

82: Fowl Adenovirus Strain Identification Challenge
Christophe Cazaban, *Ceva Animal Health*

83: Retrospective Study of Tumors of Unknown Origin in Broiler Breeders in the USA (2011-2020)
Baxter Elliot, *North Carolina State University College of Veterinary Medicine*

84: Diverse Single-stranded DNA Viruses Identified in Chicken Buccal Swabs
Darrell Kapczynski, *USDA-ARS-Southeast Poultry Research Laboratory, Athens, Ga*

85: Understanding the Respiratory virome of backyard poultry in Minnesota
Anita Kumari, *University of Minnesota*

86: Pathogenicity Evaluation of a Turkey Coronavirus Isolate (TCoV NC1743) in Turkey Poults for Establishing a TCoV Disease Model
Qingzhong Yu, *USDA/ARS/USNPRC*

Wealth of Knowledge

87: Reduction of Chondronecrosis with Osteomyelitis Lameness in Broilers Fed Metal Amino Acid Complexes Using Two Challenge Models
Raquel Konrad Burin, *Zinpro*

88: Microbiota of the poultry litter beetle (Alphitobius diaperinus)
Teresa Dormitorio, *Auburn University*

89: Why Did the Student Cross the Road: Influencing Factors to Become a Poultry Veterinarian
Linda Flores, *Western University of Health Sciences*

90: Women Pioneers in the Poultry Field 1900-Present
Jessica Hockaday, *Hockaday Consulting*

91: Biosecurity and supporting measures to improve its implementation in poultry farms
Alessandra Piccirillo, *University of Padua*

92: Health challenges and interventions in small flock poultry and waterfowl
Hailey Quercia, *Auburn University*

93: Biosecurity as a Promising Tool to Reduce Antimicrobial Use in Poultry Farms
Giuditta Tilli, *University of Padua*

Welfare

94: Understanding stress and welfare of laying hen pullets using stocking density and feeder space stressors
Meagan Abraham, *Purdue University*

95: The Influence of Mirrors as Environmental Enrichment on Pullet Behavior
Grace Sims, *Auburn University*

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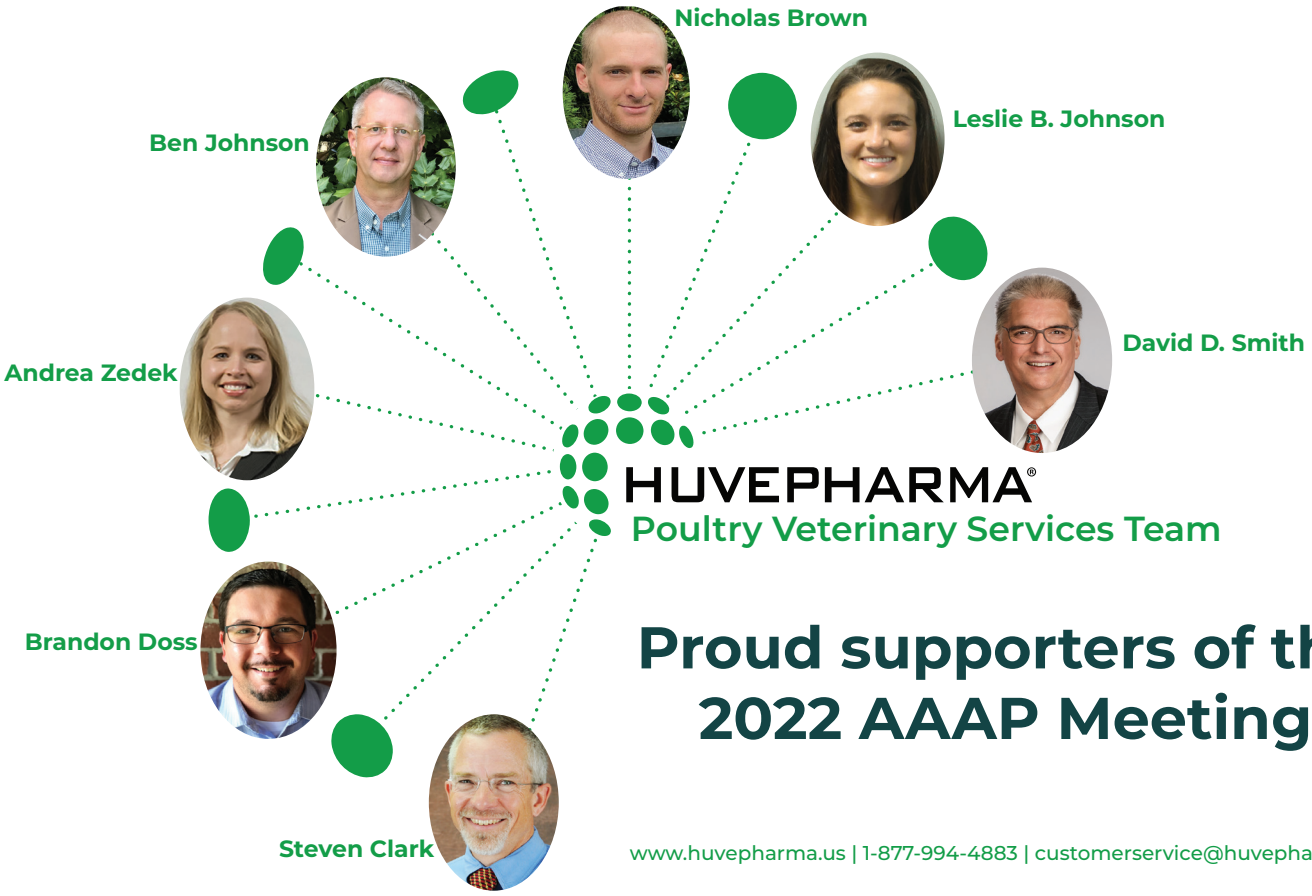

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AAAP facilitates member collaboration to advance science-based knowledge, expertise, and education on poultry health, welfare, and food safety.

AAAP Symposium

Effects of House Environment Management on Respiratory Health

Guillermo Zavala
Avian Health International, LLC

Respiratory disease is often of primary origin, albeit it can be promoted, enhanced, or complicated by inadequate poultry house conditions. Primary environmental factors directly or indirectly associated with respiratory disease include respirable particulate matter or house dust, ammonia gas and thermal stress. The anatomy of the avian respiratory system displays some unique characteristics that may facilitate pathogen access to the deep respiratory tissues. First, the lungs are rigid and cannot expand as they do in the mammalian system. Second, the air follows a unidirectional flow through the lungs, whereas air flow is bidirectional in mammals. That is, air in mammals reaches the pulmonary alveoli, which are the gas exchange unit, to then exit the respiratory tract in the exact opposite direction. Birds inhale air through the nostrils, and onto the nasopharynx, larynx, trachea, extra- and intra-pulmonary primary bronchi into the lungs. During inhalation, at least 50% of the air bypasses the lungs and is directed into the abdominal, anterior, and posterior air sacs. The rest of the air traverses the lung cranially and unidirectionally through the parabronchi, also known as tertiary bronchi. Upon exhalation, the air that went directly into the abdominal and thoracic air sacs is expelled through the lungs, allowing for gas exchange in the parabronchi. The parabronchi exhibit a central lumen from which air is directed into the atria and finally into the infundibula, where gas exchange occurs. Air moves thereafter back into the main airways to be exhaled back into the trachea. Although macrophages are certainly recruited after pathogen invasion, the avian lung seems to lack resident macrophages, which makes the innate immune responses particularly dependent on heterophils and antigen internalization by epithelial cells in the lung. Acquired immune responses in the lung are highly dependent on the bronchial associated lymphoid tissue (BALT). One of the primary risks for infection in birds is the direct access that air and PM have to the deep tissues of the lower respiratory tract.

New Developments on Mass Vaccine Application Methods

Brian Jordan
The University of Georgia

Broiler chickens are typically vaccinated by mass spray in the hatchery prior to being sent to farms and may be field boosted via spray or drinking water application between two and three weeks of age, though field boost vaccination in broilers has declined over the last 5 years. Layer and breeder pullets have traditionally been first vaccinated at ~2 weeks of age in the pullet house via spray or water, and then at multiple time points in the pullet house via spray or drinking water with live-attenuated vaccines. Killed vaccines are then given toward the end of the grow-out period and potentially into the lay period. Recently, some layer companies have moved the first IBV vaccination into the hatchery in an attempt to combat early IBV infection which can lead to false layer syndrome. As with anything in poultry production, these programs will vary by company and complex but a constant through all operations is spray vaccination, particularly in the hatchery. Interest in my laboratory in analyzing spray vaccination began by evaluating why the Arkansas type IBV persisted in commercial poultry when Ark-type IBV vaccines were commonly used to prevent it. While the vaccination process was not the main cause of the Ark IBV vaccine failures, we did realize that parts of the application equipment were detrimental to the vaccines and there were common mistakes made during the vaccination

process. Generally, there are three major places in the hatchery vaccine preparation and application process that can negatively impact the vaccine: preparation/mixing/handling the vaccine, action of the syringes, and size of the spray nozzles. Field spray vaccination introduces a few more variables, but the same concepts hold true there as well. These individual points and how they impact vaccination success as well as how they impact each other when changes are made will be discussed in this presentation.

Discussion Panel: Current problems associated with infectious bronchitis (IB)

Dr. Phil Stayer, *Sanderson Farms*
Dr. David French, *University of Georgia*
Dr. Carl Heeder, *Mountaire Farms*
Dr. Deirdre I. Johnson, *Perdue Farms*
Dr. Charles Corsiglia, *Foster Farms*
Dr. Tyler Gamble, *Pilgrims*

This panel discussion will involve six experts in poultry health, sharing their experiences in six IBV related topics:

-New molecular diagnostic tools allows us to monitor live attenuated vaccine application in the hatchery and the field. Based on recent data it appears we could be doing a much better job of handling and applying IBV vaccines. What are the most important points to consider when applying live attenuated vaccines by spray in the hatchery and in the field.

-Homologous IBV vaccines to the circulating field strain are long known to be the best at protecting against infection and disease. But when homologous vaccines are not available, what is considered acceptable cross-protection when several different IBV types are used to vaccinate birds.

-When relying on IBV vaccine cross-protection to protect birds from a unique/variant field virus, often times we see protection from disease but not from infection. What are the concerns when an apparently healthy vaccinated bird is infected with a field virus?

-IBV moves fast and one of the challenges is getting a vaccine licensed quickly for the IBV type causing problems in the field. Over the past several years, autogenous live attenuated vaccines are being developed and used because they can be licensed relatively quickly. Are there dangers associated with using autogenous live IBV vaccines? And what can be done to more quickly license safe and efficacious live attenuated vaccines for IBV?

-Molecular based type specific diagnostic tests for IBV (real time RT-PCR) were designed to detect vaccine type viruses and rely on identifying the presence of a unique sequence in the S1 gene. Is there a concern that those type specific tests will miss minor-subtypes of IBV field viruses and is that more of a concern than the potential to miss vaccine virus types?

-NGS s becoming cheaper and more readily available to individual diagnostic laboratories. What are the advantages of obtaining full genome sequences of IBV isolates in a diagnostic laboratory setting?

MY EXPERIENCES WITH FOWL CHOLERA

Mark C. Bland
Cutler Associates International

For this presentation, I will not spend much time on repeating information that is already documented in Poultry Disease books and AAAP slide sets regarding the disease itself, other than mentioning the basics as reminders. What I willdo isfocus on my experiences in dealing with Fowl Cholera and share some of the few highs and lots of lows when coming faceto face withCholerainfections in commercial turkeys, ducks and cage free layers. Iwill include examples of several cases, their outcomes (good & bad), as well as management practices, prevention strategies,

treatmentsandvaccination protocols used with cage free layers.Due to economic issues, extreme loss of birds, extra handling of birds to vaccinate cage free layers, the emphasis should be on not allowingFowl Cholera onto the farmin the first place. Biosecurity maybe importantin preventingthe introduction of Avian Influenza and Exotic Newcastle Disease onto a poultry farm, but biosecurity is the key to Fowl Cholera.

Updates on infectious coryza

Rodrigo A. Gallardo
Professor in Poultry Medicine, University of California, Davis, School of Veterinary Medicine

Infectious coryza is a respiratory disease caused by a fastidious gram-negative bacterium that has been causing severe outbreaks in temperate regions of the U.S. In addition, it has been detected in some regions without a history of being endemic. The clinical picture of the disease varies if the bacterium is accompanied by complicating respiratory agents that synergistically aggravate the clinical outcome and extend the disease course. While transmission is mostly caused by direct contact with infected or carrier birds, biosecurity breaks have a huge importance in the introduction of the bacterium to poultry flocks. Even though, still not clear there are indications that the bacterium might aerosolize and be transported by the wind or that it can be preserved by water. Results from experiments on bacterial persistence in the poultry environment and water informed that while the bacterium does not persist in bedding material it does quite well in water, especially when that water is at refrigeration temperature. In addition, chlorine at 3 ppm total, is able to inactivate this pathogen quickly or at contact.

Major Respiratory Issues of Turkeys

Eric Gonder
Consultant

This is a clinical presentation describing the effects and control of bordetellosis, Ornithobacterium rhinotracheale infection, aspergillosis, AMP-1, mycoplasmas, metapneumonvirus infection, non-H5/H7 influenzas and miscellaneous respiratory infections of turkeys. It is based on the author's experience, with advice from valued colleagues. Detailed information on the various etiological agents and pathologies will not be presented. Those desiring that information are advised to consult the appropriate sources. While biosecurity is an important in the control of some of these diseases, operational structure and environmental conditions contribute to some and will be discussed in brief detail, as will some of the long-term effects on individual birds and the affected flocks.

Discussion panel: Current problems associated with infectious laryngotracheitis (ILT)

Dr. Louise Dufour-Zavala, *Georgia Poultry Lab*
Dr. Mark Burleson, *Wayne Farms*
Dr. Donald Ritter, *Poultry Business Solutions LLC*
Dr. Nancy Reimers, *Cutler Associates*

This panel discussion will involve experts in poultry health diagnostics and epidemiology from sharing their expertise and opinions on 6 main topics:

-Recombinant ILT vaccines have already been in use for 15 years. From a field perspective and different production types (broilers, commercial layers and breeders), how beneficial have these vaccines been in controlling the disease?

-During cases of ILT in your facilities (broilers, breeders and layers), can you briefly describe the measures taken to avoid spreading the virus within the facility? Which are the measures you

consider more relevant and compelling to prevent the spread of the virus?

-ILT tends to be endemic in some regions while is not such a problem in other areas. If you have flocks in both places, what are the main differences in managing those flocks?

-Rapid diagnosis of ILT is accomplished mainly by histopathology and real-time PCR. In your experience, what decisions should be taken based on a positive ILTV result?

-Does sequencing of outbreak-related viruses to differentiate between vaccine strains and field viruses, or to identify emerging viruses add value to the diagnosis? How relevant is this information for the control of the disease?

-Is there a need for improvement of the current ILTV vaccines? If yes, what aspects of the live attenuated vaccines (CEO and TCO) and the recombinant vaccines can be improved?

AAAP Scientific Program

Keynote Speaker

Poultry, Salmonella, Campylobacter, and Food Poisoning

Douglas L. Fulnechek
Zoetis

U.S. public health agencies say that Salmonella and Campylobacter are two of the top five causes of foodborne illness among Americans. In 2010, public health officials initiated a 10-year national disease prevention program called Healthy People 2020. One of the program's goals was reducing the prevalence of foodborne illness, but that goal was, unfortunately, not met. USDA's Deputy Under Secretary for Food Safety Sandra Eskin said it's time to rethink the approach to foodborne illness prevention. The Food Safety and Inspection Service (FSIS) is mobilizing a stronger, and more comprehensive effort to reduce Salmonella illnesses associated with poultry products. FSIS recognizes that foodborne illness pathogens originate on the farm. Which is why FSIS wants the broiler and young turkey industry to adopt verified preharvest interventions to reduce the risk of food poisoning. Coordination between live production and processing plant managers is essential. Seemingly small changes in live operations can have a huge impact on FSIS sampling results at the processing plant, on a poultry company's brand, and on public health.

Avian Influenza

Global Evolution and Intercontinental Spread of H5Nx clade 2344b High Pathogenicity Avian Influenza Viruses since 2020

David Swayne
US Department of Agriculture

Evaluation of Licensed and In-House Vaccines Against Recent H5 Low Pathogenicity Avian Influenza viruses

Miria Criado
University of Georgia

Low pathogenicity avian influenza virus (LPAIV) of H5 and H7 subtypes can mutate into highly pathogenic avian influenza virus (HPAIV). Vaccination strategies are essential as a control tool to avian influenza viruses (AIV). Recently, the H5N2 LPAIVs have been isolated from poultry in the Dominican Republic (DR), raising concerns about the risk of mutating into HPAIV. Thus, our study evaluated the efficacy of licensed and in-house produced vaccines against recent H5N2 LPAIVs. Three-weeks old chickens were vaccinated with two inactivated in-house prepared from the DR- A/Ck/DR/18-44726-13/2018 H5N2 or A/turkey/Wisconsin/1968,

H5N9 and two commercially available vaccines. After three weeks, birds were challenged against two H5N2 LPAIV isolates [A/Ck/DR/19-020695-1/2018 (DR1-19) and A/Ck/DR/19-020695-11/201 (DR11-19)]. No clinical signs or mortality were observed within 14 days post-challenge (dpc). Oral (OP) and cloacal (CL) swabs collected on 2, 4, 7, and 10 dpc showed predominant viral shedding in the respiratory tract for both challenges. No differences were observed in the shedding patterns between vaccinated and non-vaccinated chickens. However, vaccination decreased the days and number of birds shedding from the gastrointestinal tract after the challenge with the DR1-19. All chickens had HI antibodies detected pre- and -post-challenge when using the challenge LPAIV as antigen, except for the non-vaccinated chicken pre-challenge sera. Therefore, our study demonstrated the low protective efficacy of in-house and commercially available vaccines against recent DR H5N2 LPAIV, reinforcing the importance of updating vaccines seed strains. Thus, it is essential to continue strict epidemiological surveillance to develop better strategies for prevention and control.

Effect of co-infection of low pathogenic avian influenza H9N2 virus and Avian pathogenic E. coli on H9N2 vaccinated commercial broiler chickens.

Wael Elfeil
Suez Canal University

For decades, low pathogenic avian influenza virus (LPAIV) subtype H9N2 has been endemic and still in many Middle Eastern countries including Egypt. The majority of losses associated with the H9N2 virus come from complicated and mixed infections in commercial broilers especially E-Coli infection. Furthermore, the role of H9N2 vaccination in worsening these losses was not evaluated before. In this work, 688,065 Arbor acres broiler chickens from the same breeder company were distributed equally in four stations, where two pens were vaccinated against LPAIV of subtype H9N2 virus, and the other two pens served as a non-vaccinated control. All were placed in the same station under the same management conditions. Twenty birds from each pen were moved to Biosafety level -3 chicken isolators (BSL-3) on days 21 and 28 of life and challenged with LPAIV-H9N2 or E-coli. Seroconversion for H9N2 was evaluated before and after the challenge. The recorded results revealed a significant decrease in clinical manifestations, and virus shedding in terms of virus amounts and the number of shedders in vaccinated birds compared to non-vaccinated birds. However, no significant differences were found between the vaccinated and non-vaccinated flocks in mortality rates. In groups co-infected with E. coli and the wild strain of LPAIV of subtype H9N2, mortality rates were higher than those in groups challenged with LPAIV of subtype H9N2 alone. In conclusion, use of the LPAIV H9N2 vaccine can minimize the losses and risks after co-infection with E-coli and AIV-H9N2 virus. Vaccination with LPAIV-H9N2 has considerably impacted the health status, amount of virus shed, and mortality of challenged commercial broilers.

Development and Characterization of a Non-transmissible H5N2 Live Attenuated Avian Influenza Virus Vaccine Based on Altered Viral M2/M42 Ion Channel Expression That Protects Against Highly Pathogenic Virus Challenge

Darrell Kapczynski
USDA-ARS-Southeast Poultry Research Laboratory

Current global outbreaks of H5Nx highly pathogenic avian influenza viruses (HPAIV) have been reported in poultry and wild birds since the emergence of the Asian Goose/Guangdong lineage of HPAIV H5N1. These detections are continuing with constant frequency. Vaccines have been developed as countermeasures to protect commercial and non-commercial poultry

flocks. Because virus antigenicity constantly changes, vaccine candidates to prevent morbidity and mortality are a moving target. The AIV M2 transmembrane protein has been demonstrated to play several key roles in virus replication. It acts as an ion channel that allows for virion acidification for uncoating and mediates virus assembly, budding and release. A variant of M2, identified as M42, was recently identified with an altered ectodomain that can functionally replace M2 in the viral lifecycle. In these studies, we tested the effects of disrupting expression of the M2 viral ion channel. Both ?M2 and ?M42 versions of the A/chicken/Pennsylvania/1/83 H5N2 virus replicated well in vitro and in ovo, but showed altered virion morphology, with the ?M42 virus exhibiting filamentous budding. The ?M2 and ?M42 virus also infected chickens efficiently and stimulated robust immune responses; however, unlike the wild-type virus, they did not transmit to naïve-contact birds. In protection studies, vaccination of birds with ?M2 or ?M42 vaccine viruses protected 100% birds from homologous and heterologous HPAIV challenge. Taken together, these studies demonstrate a strategy for developing a non-transmittable but highly protective live attenuated virus for AIV.

Phylogenetic analysis of H5N1 clade 2.3.4.4b HPAI viruses in wild birds and poultry in US in 2022

Sungsu Youk
U.S. National Poultry Research Center, ARS-USDA

Stepwise adaptation of Hemagglutinin of clade 2.3.4.4 H5N2 Highly pathogenic avian influenza virus in chickens

Investigation of a Low Path Influenza Outbreak on a Turkey Breeder Farm

Marion Garcia
Hybrid Turkeys

A turkey breeder hen flock became infected with LP Influenza after lighting but prior to production. The breeder farm is composed of 5 laying hen barns and one tom barn. The birds were moved to the lay facility over a series of days ending on 19 Nov 2021. On 29 Nov 2021, the farm manager noticed a significant drop in water consumption and quiet, inactive birds in one barn. Tracheal swabs were positive for non H5 non H7 influenza. At this time, we do not have characterization.We are following the progression of this virus on the farm as well as on our other farms in the region. This area is not densely populated with poultry but there is hog production nearby and there are a significant number of wild geese flying through. The virus will be characterized molecularly, the impact on production and flock parameters will be recorded, and the epidemiology will be investigated.

Dust as a Vehicle and Surveillance Sample of H5N8 Highly Pathogenic Avian Influenza Virus (Clade 2.3.4.4b) in Poultry Farms, France, 2021

Jean-Luc Guerin
ENVT, University of Toulouse

During winter 2020-2021, 492 avian influenza H5N8 highly pathogenic (HPAI) outbreaks have been reported in France, mostly in the South-West. After the 2016-2017 epizootic, this novel episode raises numerous questions regarding viral transmission routes and the need for new methods allowing early, easy-to-perform and affordable, detection of outbreaks. Even though wild aquatic birds are considered as the main source of initial viral introduction in commercial poultry, both inter- and intra- animal houses transmission routes are still poorly understood and ultimately poorly handled. Environmental sampling on H5N8 HPAI suspected or confirmed outbreaks were realized in order to assess the virus dispersion and potentially, develop new surveillance sampling strategies. From December 2020 to April 2021, 63 poultry houses

(either ducks or chickens) were included in the survey. Dust was sampling using wipes and aerosol sampling was performed with 2 different devices. In parallel, tracheal swabbing was performed in the framework of official surveillance. All samples were analyzed using official molecular methods and the results were analyzed using a latent class model, to assess the sensitivity and specificity of these sampling methods. Environmental sampling (either dust or aerosols) resulted in consistent PCR HPAIVs detection and showed an even higher sensitivity than tracheal swabs, especially in early stages of infection, when no clinical signs were observed in birds. Beyond their potential application for surveillance, these data confirm that dust may play a significant role as vehicle of HPAIVs, both within and between farms.

Temperature and pH stability of low and high pathogenicity avian influenza viruses

Christina Leyson
Southeast Poultry Research Laboratory

Avian influenza viruses (AIV) can be classified by disease severity in gallinaceous species as low (LPAIV) or high (HPAIV) pathogenicity avian influenza viruses. The acquisition of a multi-basic cleavage site (MBCS) in the hemagglutinin (HA) gene is the hallmark mutation that allows an AIV strain to transition from LPAIV to HPAIV. It has been proposed that HPAIVs are less environmentally stable than their LPAIV counterparts, but empirical tests are lacking. Indeed, most environmental stability studies have involved LPAIVs only, typically within the context of wild bird AIVs and their habitat. We thus compared the environmental stability between pairs of AIV strains that only differ in the presence or absence of an MBCS in the HA gene. A series of virus stock dilutions were subjected to pH 4.5-7.5 or temperatures ranging 25°-50°C. The survivability of AIVs were then measured by virus isolation in embryonated eggs. The results of this study have implications for the transmission of HPAIVs in the field and may help explain why certain HPAIVs do not transmit as well under experimental conditions compared to poultry adapted LPAIV.

Addressing common obstacles to bird transfer movements during a highly pathogenic avian influenza outbreak – a cross-commodity approach

Rosemary Marusak
University of Minnesota

During an outbreak of HPAI, the transfer movement of birds to another premises is often seen as too high stakes to even consider. This perception exists because, even when the risk that birds are infected is low, the consequences of unintentionally moving infected but undetected birds to another poultry site is high. However, delaying or canceling a bird movement creates welfare and economic issues that threaten business continuity as much as the outbreak itself. The University of Minnesota's Secure Food Systems team has created a Cross-Commodity Workgroup to evaluate the risk of moving live birds from a monitored premises to any site other than to slaughter. These bird transfer movements include broiler breeder pullets?lay, turkey breeders?lay, turkey poults?grow, and layer pullets?egg farms.The Secure Poultry Supply Plans expect premises to follow harmonized guidelines involving enhanced biosecurity in order for poultry products to move. Live bird movement guidances are based on predetermined pathways found to substantially impact the HPAIV introduction and spread risk that are common amongst all poultry industries. Within these shared pathways, commodity-specific production efficiencies pose unique obstacles to lowering the risk of bird transfer movements. These production efficiencies, which include facility design, labor needs, and transportation logistics, affect operational biosecurity practices. For example, layer, broiler breeder pullet, and

turkey poult facilities often are large, multi-house complexes. Movements from such facilities typically involve multiple transport days/destination sites which require enhanced biosecurity mitigations for safe movement. Through workgroup analysis, we discovered that cross-commodity mitigations may be implemented for the creation of cohesive permit guidances for all poultry commodities. Several notable obstacles and their subsequent risk-lowering mitigations for the broiler breeder, turkey, and layer industries have been developed and, if successfully implemented will lower risk of live bird transfer movements during an HPAI outbreak

Comparison of the pathogenicity in chickens and turkeys of a US 2022 wild bird H5N1 HPAI virus, clade 2.4.4.4b, and two previous clade 2.3.4.4 H5N8 HPAI viruses

Mary Pantin-Jackwood
Southeast Poultry Research Laboratory

The incursions of avian influenza virus (AIV) from wild birds into poultry are a continuous concern for commercial poultry worldwide. The ability of AIVs to infect any given avian species varies widely and little is known about the process of adaptation of AIVs when infecting different avian species, including the viral genetic changes associated with host restriction and virulence. To better understand adaptation of AIVs in wild waterfowl and gallinaceous species, we examined the infectivity, pathogenicity, and transmissibility of many different viruses, including low pathogenicity (LP) and high pathogenicity (HP) AIVs that caused outbreaks in the United States from 2014-2020 (subtypes H5N2, H7N8, H7N9, H7N3), and from other ongoing outbreaks in Mexico (H5N2 and H7N3), and Europe (H5N8), in mallards, turkeys and chickens. Full genome sequences of the viruses used in these studies were compared to determine genetic changes associated with virus adaptation to each bird species. Although each virus underwent specific mutations as it adapted to a given species, we have identified some mutations or markers of adaptation common to more than one virus. These studies provide important information on the evolution of AIVs and pathways to adaptation to different avian species.

Immunology

Development of a High Interferon-Inducing Live Attenuated Influenza Vaccine for In Ovo Vaccination of Chickens

Amir Ghorbani
The Ohio State University

Vaccination of chicken embryos inside eggs (in ovo) induces early immunity in young chicks while reduces the safety concerns related to the use of live vaccines on farms. However, in ovo vaccination using live influenza vaccines severely affects the egg hatchability. The aim of this study is to develop a safe and effective high interferon-inducing live influenza vaccine candidate that can be used for vaccination of chickens in ovo. In ovo-compatible vaccine candidates were reverse-genetically engineered by either replacing the hemagglutinin (HA) cleavage site of the H7 vaccine with that of the H6 virus or abrogating the expression of polymerase acidic X (PA-X) protein. Single and double mutant viruses were then tested for their pathogenicity in 10- and 18-day-old chicken embryos. Antibody responses and protective efficacies were assessed in vaccinated chickens. The HA and PA-X mutations collectively reduced embryo lethality in 10-day-old embryos by ~70%. Vaccination of 18-day-old embryos with 100 median embryo infectious dose (EID50) of the double mutant virus significantly improved the hatchability compared to the unmutated virus (83.3% vs 36.7%). Addition of two other innate immune-enhancing mutations into the polymerase basic 2 and non-structural protein 1

of the double mutant virus further improved the hatchability to levels comparable to mock vaccination (96.7%). Vaccination with up to 10^5 EID50 of the quadruple mutant virus did not result in significant loss of hatchability while protected the chickens from a heterologous challenge at two weeks after the hatch. Furthermore, no live virus was recovered from the trachea of vaccinated chicks or surface of hatching trays at the time of hatch. Successful outcome of this study is a step forward towards development and commercialization of safe, cost-effective, and broadly effective influenza vaccines for poultry.

Systemic and mucosal lymphoid immune responses during Clostridium perfringens-induced necrotic enteritis in broiler chickens

Ravi Kulkarni
North Carolina State University

Necrotic enteritis (NE), an economically important disease of chickens, is caused by Clostridium perfringens bacteria. Although the NE pathogenesis is well-studied, the immune responses against C. perfringens are poorly understood. The present used an experimental NE model to characterize immune responses against C. perfringens isolates that varied in their virulence. Broiler chickens were infected with CP5 (avirulent), CP1 and CP18 (virulent) and CP26 (very virulent) strains of C. perfringens to evaluate expression of immune genes (IL-1b, IL-6, IFNg, IL-4, IL-13, IL-10 and TGFb) in the spleen, cecal tonsils (CT), bursa of Fabricious and harderian gland (HG) tissues. Results indicated that while CP26 induced a significantly upregulated expression of pro-inflammatory IFNg, IL-6, and IL-1b cytokine genes in CT, the transcription of anti-inflammatory/regulatory IL-10 and TGFb cytokines in bursa and HG was elevated in CP18 and CP26 groups when compared to uninfected control birds. Additionally, infection with CP5 showed a significantly reduced IL-1b gene expression in HG, while the splenic pro-inflammatory transcriptional changes were observed only in the CP26-infected chickens. A Th-2 immune phenotype, as characterized by increased IL-4 and/or IL-13, was evident in birds infected with only the virulent and very virulent strains. Furthermore, macrophage stimulation with virulent C. perfringens in-vitro led to increased cellular activation, as determined by IFNg transcription and nitric oxide production. Collectively, our findings suggest that inflammatory responses during necrotic enteritis are spatially regulated such that these responses against C. perfringens depend on the virulence nature of this pathogen as well as their ability to activate macrophages.

Characterizing Pro- and Anti-viral Mirna Regulated Pathways for Newcastle Disease Virus Replication

Abhijeet Bakre
USDA

Newcastle disease caused by Newcastle disease virus (NDV) remains an ongoing concern for the poultry industry owing to thepotential for import of virulent NDV (vNDV) strains into the United States. Current vaccines against Newcastle disease virus do not prevent/ reduce viral shedding in vaccinated birds upon infection and do not protect against heterosubtypic challenge. The objective of this research is to identify host genes / pathways that prevent NDV replication and shedding so that these targets can be harnessed to improve NDV vaccines. Host encoded non-coding microRNAs regulate multiple physiological processes in animals and plants but the role of chicken encoded miRNAs in regulating NDV replication and shedding is poorly characterized. We shortlisted chicken encoded miRNAs that are predicted to regulate important interferon (IFN) and interferon stimulated genes (ISGs) computationally. Expression of these miRNAs was transiently induced in DF1 cells using synthetic mimics

followed by infection with reporter NDV viruses expression green / red fluorescent protein. Impact of miRNA over-expression on viral replication was monitored via fluorescence, hemagglutination assays and infectivity assays in embryonated SPF eggs. Host genes targeted by these miRNAs were validated using quantitative real-time PCR (qRT-PCR). These data identify novel intervention targets to reduce / mitigate NDV replication and shedding.

How Many Antigens Can You Give a Pullet: A Field Study Assessing How Multiple Vaccines Influence Antibody Titers

Gunnar Dunnam
Mississippi State University

There are conflicting reports in the literature on whether simultaneous administration of antigens leads to a decrease in humoral response compared to individual administration of an antigen. The authors wished to evaluate the effect that multiple simultaneous vaccines have on the antibody response as determined by ELISA in Ross 708 broiler breeders in a commercial setting. Sixteen-week-old Ross 708 pullets (male and female) were raised according to current company practices until pullet handling vaccination at 16 weeks of age. The miss-sexed males in each house were randomly assigned into 1 of 7 treatment groups which consisted of receiving exclusively viral vaccines, bacterins, or a combination of both. Female pullets receiving the company's standard vaccine protocol served as positive controls while one group received no killed vaccines and served as negative controls. Birds were bled 32-33 days after vaccination. BioChek ELISA kits were used to measure antibodies versus Newcastle disease, infectious bronchitis, bursal disease, reovirus, fowl adenovirus, and Salmonella B and D. Statistical differences among groups were determined by non-parametric methods. Virus neutralization is being conducted on fowl adenovirus titers with analysis to follow. There was no significant negative effect on the antibody levels versus all antigens assessed when the birds received all the vaccines at the same time. With exceptions, the addition of vaccine treatments that lacked the antigen being measured seemed to decrease titer level numerically but not significantly. There was also evidence that the addition of two vaccines with the same antigen did not raise titer levels significantly.

Vaccinology

Pasteurella multocida Vaccines with Lower or No Post Vaccination Reaction Adjuvants in a Fowl Cholera Challenge Model

Charles Hofacre
Southern Poultry Research Group, Inc.

Broiler breeders are often vaccinated in the pullet house with either live attenuated or inactivated vaccines. The inactivated vaccines with oil emulsion adjuvants have the advantage of a strong immune response affording protection to fowl cholera but the post-vaccine reaction causes many pullets to stop eating thus affecting growth resulting in body weight uniformity, ultimately affecting egg production. This study evaluated an autogenous inactivated serotype 1 antigen with four different adjuvants: Two commercially available adjuvants: Emulsigen D and Emulsigen-P plus two experimental adjuvants: 111 and 222. Sixty Ross 708 broiler breeder pullets per treatment were reared per breeder guidelines to 35 days of age and then individually wing banded and vaccinated in the left breast muscle with 0.5 ml vaccine or PBS. Ten birds were injected with antigen only without adjuvant. On day 49 each bird was boosted with same vaccine or PBS in right breast muscle. On day 63(14 days post

boost), ten birds per treatment were euthanized and vaccine reaction externally and internally scored (0-4); cumulative score ranging 0 to 32. All remaining 50 pullets/treatment were challenged by intramuscular injection of the USDA challenge strain serotype 1 (1.5 x 103 CFU/bird). The challenge control (PBS) had mean lesion score results of 0.3; antigen only control (0.0); Emulsigen-D (1.7); Emulsigen-P (1.6); adjuvant 111 (3.0) and adjuvant 222 (2.4). These lesion scores were extremely low compared to lesions observed by the author in previous studies with oil emulsion adjuvant vaccines. Body weight gains were similar between treatment groups. Fowl cholera mortality post challenge was 100% in the challenge control; 64% Emulsigen-D; 60% Emulsigen-P; 32% adjuvant 111 and 44% adjuvant 222. This study demonstrated that the new polymer based adjuvants can be candidates for effective vaccines in preventing fowl cholera mortality while not causing a severe vaccine reaction at the intramuscular injection site in broiler breeder pullets.

Experiences with a safe live Pasteurella multocida vaccine

Chris Morrow
Bioproperties

Recent advances in understanding of the LPS structure of PM isolates has explained the limitations of Heddleston serotyping in predicting protection by killed vaccines. There are 8 recognised LPS genotypes with the main chicken genotypes being in either L1 (Heddleston serotypes 1 and 14) or L3 (Heddleston serotypes 3, 4 and 3/4) or L6 (Heddleston serotypes 10, 11,12, and 15). Obviously just getting the result L1 from a farm investigation can tell you that if you are using a L3 killed vaccine that it will probably not work (but gives no guarantee that a L1 killed vaccine will work). Even within a Heddleston serotype some variations in LPS structure will be not generate homologous protection. This explains why autogenous vaccines are initially successful, but the field strain may change by becoming an escape mutant. There is some evidence emerging that this ability to change LPS structure (within a LPS genotype) may be an innate property of PM strains used for immune evasion during natural challenge. Antibody responses to killed vaccines are generated to LPS antigens remaining immunogenic after inactivation. Late breaks in lay in autogenous vaccinated flocks may be due to duration of immunity being insufficient and may be able to be predicted by antibody levels. This has been further complicated by the observation of LPS genotype switching (Omaleki et al., Microbial Genomics 2020;6 DOI 10.1099/mgen.0.000346) in a field strain challenging a farm (a MLST ST20 clone going from L3 to L1 genotype) or two field strains with the same ST but different L types.Live PM vaccines generate broad immunity within and across LPS types and probably includes CMI. The current problems with traditional live vaccines are reversion to virulence or residual virulence. In Australia we have been using an AroA mutant PM vaccine by injection (Parent strain X95 US Heddleston serotype 1 type strain). This safety attenuation is particularly effective in Chickens (but not Turkeys) as this strain (PMP-1) only multiplies in the laboratory and not in the bird or the environment after exhaustion of aromatic amines and cannot be found in the chicken after 2 weeks. Duration of immunity in the lab to homologous challenge and heterologous challenge has been limited but in the field seems to be adequate (the laboratory challenge models are very strong. This is efficacy in the laboratory. What we need now is efficiency in the field reports).

Vaccination Trials in a Midwestern Heavy Tom Complex Using a Clostridium septicum Bacterin/Toxoid

Andrew Smith
Butterball

Gangrenous dermatitis caused by C. septicum leads to increased mortality, production losses, and antibiotic usage in heavy toms, making it one of the most important health issues facing the turkey industry. With pressure to limit antibiotic use, vaccination may be needed to prevent gangrenous dermatitis in the future of turkey production. This presentation will give an overview of field trial data using an injectable C. septicum bacterin/toxoid in a midwestern production complex growing heavy toms.

Development of a Novel Vaccine Strategy Against Necrotic Enteritis by Synergizing Immune-enrichment and Immunostimulation in Broiler Chicks

Hemlata Gautam
University of Saskatchewan, Canada

The withdrawal of prophylactic antimicrobials use in the chicken industry has led to a substantial increase in various bacterial infections, including Clostridium perfringens (CP). In this study, we aimed at developing a novel vaccine strategy against CP by synergizing immune enrichment with immunostimulation in broiler chicks. The experiment was conducted using four groups (n=35/group), 1- Controls, 2- CP challenge only, 3- In ovo CpG-ODN + inactivated CP vaccine, and 4- Non-CpG-ODN + Inactivated CP vaccine. The vaccinated birds received a booster dose at day 10 of their age. Groups 2, 3, and 4 were challenged with CP via feed (feed: media, 1:1) at days 20, 21, and 22, twice daily. Blood, intestinal mucosal scrapings, and tissues (for histopathology) were collected at the trial end. The serum IgY, intestinal mucosa IgA, and protection from CP challenge (as evidenced by low histopathological lesion scores) were significantly high in group 3 compared to controls.

Effect of Coccidial Vaccines on the Stability of Salmonella and Viral Live Attenuated Vaccines

Alejandro Banda
Mississippi State University

Studies to describe the impact of mixing different coccidial vaccines on the stability of infectious bronchitis (IBV), Newcastle disease (NDV) vaccines, and the stability of a Salmonella Typhimurium live vaccine were conducted. In experiment 1, one monovalent (Mass), one bivalent (Mass+Ark) IBV, and a NDV vaccine were titrated alone or in combination with coccidial vaccine A at different time points after reconstitution and mixing. No negative effect on the titer of the three vaccines was observed in combination with coccidial vaccine A, only minimal reduction in NDV vaccine titer at two and three hours when combined coccidial vaccine A. In experiment 2, the stability of a live attenuated infectious bronchitis virus (IBV) Mass type and S. Typhimurium vaccines in combination with three different coccidial vaccines: coccidial vaccine A, vaccine B, and vaccine C (with potassium dichromate) was evaluated by titration at different timepoints after reconstitution and combination. Minimal titer reduction of IBV vaccine was observed in combination with cocci C after two hours at room temperature. However, the Salmonella vaccine showed significant titer drops after two hours (1 log10), and four hours (3 log10) when combined with cocci vaccine C. Experiment 3 (in vivo study) was conducted in one-day old chicks spray vaccinated with Salmonella vaccine in combination with coccidial vaccine A, and vaccine D (with potassium dichromate). The Salmonella vaccine was re-isolated from chicks that received the combination with cocci vaccine A, but no re-isolation was obtained from chicks vaccinated with combination with coccidial vaccine D.

Adenovirus Progeny Protection Trial in Breeders

Emma Castillo
Aviagen North America

In this progeny protection trial, two breeder flocks were vaccinated with AviPro autogenous fowl adenovirus vaccine containing serotypes 8a, 8b and 11. The breeders' progeny were then challenged with adenovirus 8a, 8b and 11 and assessed for disease. Three individual studies were performed as part of this trial.The first study established pathogenicity of field adenovirus 8a, 8b and 11 isolates using specific pathogen free chickens (SPF). The SPF chickens were split into eight groups of ten chicks. The first four groups contained a negative control, a group challenged with FAdV 8a, a group challenged with FAdV 8b and a group challenged with FAdV 11. All challenges occurred at three days of age. The remaining four groups were split into the same categories but challenged with adenovirus at 11 days of age. Pathogenicity was successfully demonstrated 72 to 96 hours post exposure with no differences seen between the days of challenge.The second study assessed the breeder flock's adenovirus antibodies using BioCheck FAV1 adenovirus enzyme-linked immunosorbent assay (ELISA) and adenovirus virus neutralization (VN). A subsection of the progeny were assessed for adenovirus maternal antibodies using VN and compared to their source flock antibodies for correlation. Discrepancies between one source flock and the progeny required a closer evaluation.In the third study the two breeder flocks' progeny was split into 4 groups each and challenged similarly to the specific pathogen free chickens. No clinical signs or disease was seen on histopathology for either of the breeder flocks' progeny. This study establishes the efficacy of the autogenous vaccine and takes a closer look at adequate breeder adenovirus antibody values.

Protection of Ultifend® & Rispens vaccine against Infectious Bursal Disease Virus Variant E Challenge

Olivia Faulkner
Ceva

A vectored vaccine containing turkey herpesvirus (rHVT) with dual insert of infectious bursal disease (IBD) and Newcastle disease (ND) antigens, referred to here as Ultifend®, was developed in combination with Marek's disease virus, Serotype 1 (Rispens). Previous studies demonstrated that Ultifend® & Rispens vaccination by subcutaneous (SQ) route in specific pathogen free (SPF) chickens was efficacious against homologous infectious bursal disease virus (IBDV) standard strain challenge. For this study, cross protection of the IBDV standard strain virion protein 2 (VP2) antigen in the vectored vaccine fraction of Ultifend® & Rispens was evaluated in SPF chickens against IBDV Variant E challenge. The objective of this study was to evaluate whether SQ administration of Ultifend® & Rispens was effective against disease caused by IBDV Variant E strain. Chickens were vaccinated on the day of hatch, and challenged with IBDV Variant E strain at 5 weeks old. For a successful study, at least 90% of the Ultifend® & Rispens vaccinated chickens (n=30) must remain free from gross IBD lesions and at least 90% of the positive control group (n=30) showing gross IBD lesions. In the group vaccinated with Ultifend® & Rispens, 28 out of 30 chickens (93%) were protected against the IBDV Variant E challenge based on the presence or absence of gross lesions; while in the unvaccinated control group, 28 out of 30 chickens (93%) had IBD gross lesions that were consistent with the IBDV Variant E challenge. These results suggest that SQ administration of Ultifend® & Rispens was protective against IBDV Variant E challenge.

Reovirus

Genetic and Antigenic Relatedness of Avian Reovirus Variants

Sofia Egana
UC Davis

Avian reoviruses (ARVs) are in constant evolution and, because of that, vaccination strategies should consider the homology between field challenge viruses and autogenous or commercial vaccines. In addition, the current ARV molecular classification method is not accurate enough to determine antigenicity and consequently cross-protection between strains. To ensure optimal vaccine selection, it is important to associate genetic diversity with the antigenicity of variant strains. In previous experiments, we have found the highest genetic variability in genes L2, M2, S1, and S4. To understand if that genetic divergence is associated to antigenicity, we designed a genetic and antigenic cartography model. Twenty-six genetically and pathogenically diverse avian reoviruses were selected and plaque purified. Some of them were used to produce hyperimmune sera in SPF chickens. Viral cross-neutralization was performed, and the data obtained were transformed to calculate antigenic distances (AD) and plotted into an antigenic map. The genetic distances (GD) between strains were calculated based on amino acid substitutions of the relevant proteins. Finally, correlations between the AG and GD were performed by using regression analysis. The results of these experiments will be discussed.

Antigenic Variation and In Vitro Neutralization of Avian Reovirus Isolates from Mississippi

Rebecca Mackey
Poultry Research and Diagnostic Lab, Mississippi State University

Over one hundred cases with positive reovirus isolation were received at the Poultry Research and Diagnostic Laboratory (PRDL) in the past four years. The isolates exhibited genetic similarities of 50% or lower in comparison with the vaccine strain (S1133) and related strains. These Mississippi field isolates were typically isolated from three to four-week-old broilers with clinical signs that included lameness and hock joint arthritis, intestinal or size-uniformity issues, mortality, and three cases with neurological problems. The genotyping of a subset of samples by sequencing the S1 gene indicated that these isolates have representatives in all six identified avian reovirus genotypic clusters. To assess serological variance of the reovirus isolates, virus neutralization assays were performed using LHM cells. A 50% infectivity titer (TCID50) was determined for each isolate, and neutralization indexes were calculated. Microneutralization test results from antigenic variants suggested that there is some degree of cross neutralization with the S1133 strain and related reoviral strains. Correlations between genotyping and in vitro neutralization results are discussed, along with the need for a serological approach for typing avian reovirus isolates.

Genetic, Antigenic, and Pathotypic characterization of newly emerging Turkey Hepatitis Reovirus

Sunil Mor
University of Minnesota

Avian reoviruses continue to cause disease in chickens and turkeys with varied pathogenicity and tissue tropism. Recently, starting January 2019, we have reported on new variants of reoviruses causing hepatitis and mortality in turkey poults with median age of 15.5 days. The change from causing mild enteric disease to economically important arthritis and hepatitis highlights the need for genetic, antigenic, and pathotypic characterization of newly emerging turkey hepatitis reoviruses (THRVs). The selection of vaccine strains of reoviruses is challenging due to the ability of their genomes to mutate and recombine, substantial heterogeneity in the spatial distribution and

spread of different strains, and potential turnover in dominant strain across time. Understanding the evolutionary and epidemiological characteristics of THRVs is critical for designing effective vaccination campaigns at national level. Integration of THRV sequence data and epidemiological metadata in a Bayesian phylodynamic framework will help quantify evolutionary change in the virus across time, differentiate between endemic and emerging strains, and ultimately help identify appropriate strains for manufacturing effective vaccines. Seventy THRVs representing different states, age, year etc were selected for whole genome sequencing. Out of these, 30 were submitted to AviServe for serotyping and 10 were selected for comparative pathogenicity study. One-week-old turkey poults were inoculated orally with 0.2 ml (~10^5 TCID50/ml). At 3, 5, 7, 14, 21, and 28 days post inoculation samples were collected for histopathology and real time RT-PCR. The results of whole genome sequencing, phylodynamic analysis, serotyping and pathotyping will be discussed in details.

Diagnostics

Get the flu out of here…HPAIV environmental stability in outbreak waste and environmental testing strategies

Erica Spackman
SEPRL-USDA-ARS

In order to identify the best sample collection device for detecting viruses in the environment, 3,894 samples were collected from a variety of surfaces (wood, metal, plastic, etc.) in different housing types occupied by chickens experimentally exposed to avian paramyxovirus type 1, infectious bronchitis virus, or low pathogenic avian influenza virus. Five devices/procedures were evaluated: cotton gauze pre-moistened with brain-heart infusion buffer (moistened, not saturated), dry cotton gauze, factory pre-moistened cellulose sponges, foam swabs, and spun polyester swabs (same swabs that are used to collect oro-pharyngeal and cloacal samples from birds). Across all surfaces and with all viruses, pre-moistened gauze recovered significantly more virus than any of the other devices based on titer equivalents determined by quantitative real-time RT-PCR. The only other significant difference was that dry gauze recovered more virus than polyester swabs. The proportion of positive samples was significantly higher with pre-moistened gauze and dry gauze versus all other devices. The device that was consistently the poorest performer (lower proportion positive, lowest titers recovered, and a limit of detection about 10X higher than pre-moistened or dry gauze) was the polyester swab. Although more manipulation is needed with pre-moistened gauze, the increased sensitivity is a great advantage. Cotton gauze is also inexpensive and easy to find. Importantly, swabs that work well for collecting samples from birds should not be used for collecting environmental samples.

Nanopore Sequencing: A Promising Tool for Fast Identification and Accurate Characterization of IBV Directly From Clinical Samples

Brittany Skaggs
Iowa State University College of Veterinary Medicine

Infectious bronchitis virus (IBV) is a coronavirus that affects the respiratory system of chickens, causing major economic losses within the poultry industry. Like other Coronaviruses, IBV has a high mutation rate resulting in the continuous emergence of variant serotypes. There is little to no cross-protection between IBV serotypes, therefore, serotype determination is necessary for vaccine selection. The most common method of IBV serotype characterization is through sequencing the S1

gene. However, sequence typing is not always possible. Due to the high mutation rate, sequencing primers often fail to anneal and need to be continuously updated. Oxford Nanopore Technology (ONT) is a third-generation sequencing platform that utilizes sequence-independent technology to sequence nucleic acid in its native form in minutes to hours. The objective of this study is to evaluate the implementation of ONT as a diagnostic tool for identification and characterization of IBV directly from clinical samples. In this study, serial dilutions of different positive IBV clinical sample were used to evaluate ONT's ability to correctly identify IBV in comparison to qPCR and characterize IBV into its correct serotype in comparison to Sanger sequencing. Results from this study have shown that ONT is a very promising tool to complement the current IBV molecular diagnostics or potentially replace them in the future. As a result, the use of ONT can streamline the laboratory diagnosis of IBV, which will inform and expedite the vaccine selection process and facilitate disease prevention and control.

Fast Identification of Emerging Avian Viruses Using Nanopore Sequencing Device

Guillaume Croville
ENVT - University of Toulouse

Poultry health is increasingly challenged by emerging infectious threats associated with animal reservoirs and global transport of animal. The emergence of highly pathogenic avian influenza viruses is an example of infectious threats challenging global livestock sustainability.Classical molecular detection approaches are based on real-time PCR techniques targeting viral genes for a fast detection and quantification. Despite its robustness, PCR will hardly generate information on subtypes or presence of minority mutants. Propagation of viruses on cells or embryonated eggs before PCR or NGS is also a robust and efficient solution but is time-consuming and not compatible with a field emergence requiring a fast analysis.We had already tested unbiased NGS directly performed on samples to identify pathogens without previous knowledge: we were able to identify and type avian poxviruses and adenoviruses. Here, we tested workflows combining multiplex-PCR enrichment and Nanopore next generation sequencing. The Flongle flowcell associated with the MinION device from Oxford Nanopore Technologies fits perfectly with a rapid diagnostic, considering its convenience, price and data throughput.We developed a pipeline from the sample to bioinformatics analysis, allowing a complete dissection of the pathogens population in the same-day. This pipeline was applied to the detection and typing of influenza or infectious bronchitis viruses and coinfecting agents, directly sampled in the respiratory tract of infected birds. This approach allows a fast and sensitive NGS-based detection and typing of AIVs or IBV, virtually applicable to any viral disease.

This is How Today's Commercial Meat type and Egg Type Chickens Became ALV-Free

Guillermo Zavala
Avian Health International, LLC

This is How Today's Commercial Meat type and Egg Type Chickens Became ALV-FreeGuillermo ZavalaAvian Health International, LLC – Flowery Branch, GA (USA)Avian Leukosis Viruses (ALV) are the representative genus of the Alpharetroviruses. Exogenous ALVs are oncogenic viruses that are transmissible congenitally or horizontally. ALV subgroups that are particularly relevant for the commercial poultry industry include ALV-A, ALV-B, ALV-J and ALV-K. Subgroups A and B cause primarily lymphoid leukosis; ALV-K induces mostly myelocytomas, myeloblastomas, and myelomonocytic myeloid leukemia, but also a variety of additional tumors. ALV-K is associated with various tumors and has been confined to

China. All other subgroups have caused serious health problems to chickens worldwide at various times. Lymphoid leukosis caused very significant health and performance problems in egg type layers until eradication took place circa 1990. ALV-J peaked as a problem in meat type chickens between 1994 and 1998. All exogenous leukosis viruses have the potential of causing tumors, mortality, decreased egg production and a deficit in broiler performance, among other detrimental effects. Various subgroups of ALV including ALV-K continue to cause problems in China until present. ALV eradication was accomplished in Western breeds of egg type and meat type chickens by using a variety of laboratory tools, and control, reduction, and eradication strategies. This presentation summarizes the use and application of the COFAL test, antigen capture ELISA, antibody ELISA, virus isolation and molecular detection methods toward eradication of ALV. A description of the actual methods and strategies used for testing and culling of infected breeding stock at the elite level is included.

Biomarkers for Rapid Identification of White Chick Syndrome (Astrovirus) Cases at the Hatchery

Daniel Venne
Couvoir Scott ltée

It is important for hatcheries to rapidly identify cases of white chick syndrome to ensure that affected chicks are culled before getting to the growout barns because of poor performance in grow out. Since pale colored chicks can occur from different causes it is important to be able to rapidly diagnose the condition. Classical testing is done by PCR which requires a speicialized laboratory and an important amount of time. We will present difrerent biomarkers and in particular the use of a portable glucometer and Ketone strips as a rapid method for the field diagnosis of the condition.

Infectious bronchitis virus and chicken laryngotracheitis infectious virus in backyard poultry located around breeder poultry farms in Brazil

Helena Lage Ferreira
University of Sao Paulo

A suspected avian influenza virus (AIV) and Newcastle disease virus (NDV) in poultry flocks require immediate compulsory notification to the official veterinary service (OVS). However, other respiratory viruses such as infectious bronchitis virus (IBV) and infectious laryngotracheitis virus (ILTV) induce similar clinical signs in chickens. The present study evaluates the circulation of these viruses in backyard poultry flocks located around five poultry breeding farms (broiler great grandparents, grandparents, breeders, and hatcheries) in the northwest region of the Sao Paulo state, Brazil. Census and epidemiological questionnaires were carried out in the backyard poultry farms. Oral swabs were collected to detect AIV, NDV, IBV, and ILTV by molecular tests. From October 2017 to October 2018, 117 pools of 583 oral swabs from 1495 birds were collected from 76 (73.07%) out of 104 identified backyard farms. AIV and NDV were not detected in any sample, whereas IBV and ILTV were detected in 27.63% (n=21) and 9.21% (n=7) of tested flocks. The census identified four potential risk factors for the introduction and dissemination of IBV and ILTV into the farms. IBV infection was associated with 138 times ($P<0.001$) more detection of ILTV in backyard poultry farms. Obtained IBV sequences were clustered with the Brazilian strain (GI-11) based on the S1 gene. Our data shows the virus circulation in backyard poultry farms, and biosecurity measures should be strength in poultry farms to avoid virus circulation between farms.

Epidemiology

Epidemiological investigation of MS in broiler breeders using the 14 NPIP biosecurity principles

Louise Dufour-Zavala
GPLN

Several detailed epidemiological investigations were made on broiler breeder farms diagnosed with Mycoplasma synoviae using the 14 NPIP biosecurity principles during 2019-2021 in the Southeast US. The line of separation, visitor control and rodent control were the principles most often found to be lacking attention and posing a risk of disease entry. An analysis of these observations will be shared.

Development of a multilocus sequence typing scheme for Avibacterium paragallinarum

Mostafa Ghanem
University of Maryland-College Park

Avibacterium paragallinarum (AP) is a gram-negative, non-motile bacterium that causes Infectious Coryza (IC), a respiratory disease of chicken. Recently, there has been an increased prevalence of IC in commercial and noncommercial chickens. Current strain differentiation methods of AP like classical and molecular serotyping have limited value and discriminatory power to investigate the epidemiology of AP outbreaks and population structure. Therefore, a feasible and accurate strain differentiation method is needed to investigate the epidemiology of IC and to prevent future outbreaks. We hypothesized that Multilocus Sequence Typing (MLST) could provide better discriminatory power and a more accurate method for typing AP directly from clinical samples without isolation. To test this hypothesis, we have studied all AP whole genome sequences (WGS) available at GenBank and identified eighteen candidate housekeeping genes. Primers were designed and successfully amplified for all genes from a few samples including isolates and PCR positive samples. The phylogenetic relationship between all AP genomes and clinical samples was compared using MLST, Adhoc cgMLST, HPG2, and hmtp210 when sequences were available. As a result, seven gene segments were selected for the final MLST based on nucleotide diversity and discriminatory power. The MLST appeared to have higher discriminatory power compared to HPG2 and hmtp210. Moreover, more agreement was found between our newly developed MLST and Adhoc cgMLST using the tested samples. More samples are currently processed to further evaluate the newly developed scheme. To conclude, the newly developed MLST scheme represents a promising tool for strain differentiation of AP. It enables a better understanding of AP epidemiology and population structure. In addition, it provides a non-ambiguous, portable, and universal typing for AP that would facilitate prevention and control efforts worldwide.

A time-space investigation for better understanding the epidemiology of Ornithobacterium rhinotracheale in commercial turkeys in Iowa

Yuko Sato
Iowa State University

Ornithobacterium rhinotracheale (ORT) is one of the major bacterial agents implicated in respiratory diseases in US turkeys and continues to be one of the top 5 health problems in the industry for many years. While it is true that ORT can be isolated from birds with significant respiratory disease, the majority of challenge studies aiming to reproduce failed to do so, which casts significant doubts on its role as a primary pathogen. Additionally, the epidemiology of ORT in terms of its sources of infections and of transmission dynamics is still unclear and requires further investigation. Samples were collected to investigate the prevalence of ORT in birds and the environment of apparently healthy flocks as well as

investigating ORT source of infection and how it spreads among the flocks. Sequential longitudinal respiratory (tracheas and lungs) and environmental (feed, water, litter and varmints) samples were collected from commercial turkey flocks with a known history of ORT infections (case, n=2) and without a history of ORT infections (control, n=1). Samples were collected at eight time points from hatch to processing in the summer of 2020. Additionally, cross-sectional respiratory samples (tracheas and lungs) were collected from 50 different apparently healthy flocks during posting sessions held in February of 2020 and February of 2021. Collected samples were tested for ORT using quantitative real-time PCR (qPCR). Results showed that ORT was present in 24.29% of the longitudinal samples including both control and case flocks. In addition, detection of ORT occurred as early as three weeks of age with the highest prevalence between 5 to 12 weeks of age. Sixteen out of 42 flocks (38%) of the cross-sectional samples had positive qPCR results. Obtained results from this study suggests that ORT can be detected within normal turkey flocks with the absence of history or clinical signs of ORT.

Multistate Psittacosis Outbreak at Chicken Slaughter Plants— Virginia and Georgia, 2018

Christine Szablewski
CDC

Multistate Psittacosis Outbreak at Chicken Slaughter Plants — Virginia and Georgia, 2018Christine M. Szablewski, DVM1,2; Kelly A. Shaw, PhD1,3; Olivia L. McGovern,4; Miwako Kobayashi, MD4; Marie A. de Perio, MD5; Julia Murphy, DVM3; Cherie Drenzek, DVM2; Julie Gabel, DVM21Epidemic Intelligence Service, Division of Scientific Education and Professional Development, CDC; 2Georgia Department of Public Health; 3Virginia Department of Health; 4Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, CDC; 5Division of Surveillance, Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, CDCPsittacosis is a respiratory infection in humans caused by Chlamydia psittaci, a bacterium found in respiratory secretions and droppings of infected birds. In Fall 2018, a large psittacosis outbreak occurred among workers at affiliated poultry slaughter plants in Virginia and Georgia. The last psittacosis outbreak among U.S. poultry plant workers was reported in 1989. A multiorganizational team investigated the outbreak causes and risks of illness to prevent additional cases and guide worker safety practices. A case was defined as illness in a Georgia or Virginia worker with either PCR-confirmed C. psittaci infection, physician-diagnosed pneumonia, or fever or chills with =2 symptoms of headache, cough, and myalgia. A cohort study (Virginia) and a case-control study (Georgia) were performed. Environmental plant samples were tested for C. psittaci. A health hazard evaluation (HHE) was performed at the Virginia plant. Eighty cases (50 in Virginia, 30 in Georgia; 13 confirmed) were identified. Twenty-nine workers were hospitalized; 43 workers were diagnosed with pneumonia. Ever working in the evisceration area was a risk factor for illness in Virginia (Relative Risk: 2.1; 95% CI: 1.1–3.8) and Georgia (Odds Ratio: 2.6; 95% CI: 1.2–5.8). C. psittaci was not detected in environmental samples. HHE recommendations included modifying work practices to reduce contamination.This is the largest-ever psittacosis outbreak in a chicken slaughter plant in the United States. Psittacosis can cause severe illness. Exposure to the evisceration area increased worker illness risk. Recommendations to prevent illnesses focused on reducing plant worker exposure to contaminated aerosols.

Turkey Coronavirus Enteritis: Lessons and Experiences From the Field

Eric Orozco
Butterball

Turkey Coronavirus Enteritis (TCE) is a disease that affects mostly young turkeys but can also affect older birds. It causes an acute highly contagious disease that is characterized by enteritis, depression, retarded development, diarrhea, poor weight gain and feed conversion. There is currently a TCE outbreak with over 100 confirmed positive flocks in both divisions in NC, including brooder and finisher farms, since June 2021. The last outbreak was from June 2018 through June 2019 with a total of 92 positive flocks. The transmission has been observed in farms within 1-2 miles radius around positive premises and in instances of shared help and equipment. Other clinical signs such as high mortality and severe secondary infections have been observed. Different interventions for control have been put into practice, involving all the areas in live production. The aim of this study is to discuss specific characteristics of this current outbreak in regards to transmission, infectious process, diagnosis, and control.

Infectious Bronchistis Virus

IBV surveillance as a tool in a disease prevention program

Rodrigo Gallardo
University of California, Davis

nfectious bronchitis causes severe economic losses among chicken flocks worldwide. The causal agent of IB i.e., IB virus (IBV) is a highly variable virus due to the nature of its genetic material (RNA) and management practices perpetuated in the poultry industry. Epidemiological surveillance is one of the tools that should be part of every control and prevention program against IB. Surveillance efforts can educate producers in seasonality, spatial and temporal trends in addition to the most prevalent genotypes affecting a zone and the repercussion of vaccination programs being utilized. Finally, new surveillance strategies can provide early variant detection which allows for preparedness. In this presentation we will review surveillance efforts in broilers and layers their differences, similarities and their role in a good IBV prevention and control strategy.

Assessment of infectious bronchitis virus (IBV) exposure risks in broiler chicken using spatial analysis, machine learning, and risk factor analysis.

Andreia Arruda
The Ohio State University

There are growing concerns in the poultry industry regarding preventing and controlling outbreaks of emerging endemic or epidemic avian pathogens, e.g., infectious bronchitis virus (IBV). Despite biosecurity and vaccination protocols, these concerns warrant the investigation of additional methodologies to help identify risk factors for disease spread. The objective of this study was to use IBV antibody titers in combination with spatial, machine learning, and traditional epidemiological risk analyses to investigate key factors in the surrounding farm landscape linked to an increase in titer levels. Antibody titer data from 130 broiler farms of a company was obtained for 6 years (2016-2021). Spatial variables including landcover, road networks, waterways, topography, as well as the study farms and all other operating poultry farms and slaughter plants in the region were plotted in QGIS 3.18. Proximity and farm density data by commodity (layer, broiler, layer pullet or turkey) were also extracted. A Boruta machine learning algorithm was used to select proximity and density-related variables that were critically associated with increases in IBV antibody titer. Selected variables were imported into a mixed effect logistic regression model constructed in STATA 15.1. The

odds of having high IBV antibody titers levels were 2.3-4.7 times higher compared to our reference year 2017. Farms surrounded with high broiler farm density within 3-mile radius or located = 10 miles from a slaughter plant, had 30% and 34% higher odds of higher IBV antibody titers, respectively. In conclusion, herd-level IBV antibody titer could be related to the density of surrounding broiler farms. Future refinement of spatial data analysis as a risk assessment tool for local or regional poultry production areas may yield a better understanding of IBV risk in the surrounding farm landscape.

Proactive management of Infectious Bronchitis Virus (IBV) challenge in commercial broilers: Lessons learned from field cases

Matilde Alfonso
Ceva Animal Health

Proactive management of Infectious Bronchitis Virus (IBV) challenge in commercial broilers: Lessons learned from field casesMatilde Alfonsoa, Sara Throneb, Bill Hewatc, Scott Gustinc, Kurt Dobsond, Jose Linaresa, Robert Becksteade, Marshall Putnamaa Veterinary Technical Service, Ceva Animal Health, U.S.Ab Simmons Foods. Siloam Springs, AR. U.S.Ac Tyson Foods. Springdale, AR. USAd Georges Inc. Springdale, AR. USAe Scientific Support and Investigation Unit, Lenexa, U.S.AThe emergence of variant Infectious Bronchitis viruses in the USA in recent years (i.e. DMV1639, GA08, GA13) has shifted how the broiler industry is controlling this disease both through vaccination and monitoring. This paper describes a practical case using current diagnostics tools (IBV serology, IBV qRT-PCR, and IBV sequencing) to investigate and control a DVM1639 IBV infection in commercial broilers reared in a densely populated chicken area. The case was triggered by an increase in IBV ELISA titers observed during routine serological monitoring at processing. Further investigation based on IBV qRT-PCR revealed a widespread DMV1639 infection across the operation, which was unusual in the region based on historical surveillance data. IBV S1 sequencing was performed to characterize the virus in case any genetic changes had occurred. Concurrently, IBV vaccination was validated bv IBV qRT-PCR and vaccination changes were implemented to improve early vaccine replication. Follow up IBV surveillance was performed to assess the results of those changes on the level of infection. The outcome of this investigation is presented in this report. Proper use of existing vaccines and diagnostic tools is allowing the USA broiler industry to proactively manage current IBV challenges.

Comparison of Infectious Bronchitis Virus Surveillance Profiles in Layers, Broiler Breeders, and Broilers

Brian Jordan
The University of Georgia

Control of infectious bronchitis virus (IBV) is attempted by using vaccines that are mass applied in the hatchery and in the field. IBV vaccines are usually serotype specific, meaning these vaccination programs vary based on the field challenge strains of each complex. Cross protection between serotypes is limited and the need for accurately matched vaccination programs to field challenge is imperative. In order to match the vaccination program, the serotypes circulating in flocks must be identified. A current tool for IBV identification is quantitative real time RT-PCR. This assay can detect specific serotypes, as well as provide a relative viral load in each set of samples tested. A comprehensive IBV qRT-PCR surveillance program was started by the PDRC to assess what IBV serotypes were actively circulating in flocks. Samples from layer, broiler breeder, and broiler flocks were collected from multiple complexes spanning the US. Samples were tested using a universal IBV qRT-PCR assay, and then with Massachusetts, Connecticut, Georgia08, DMV/1639, Arkansas, Delaware/Georgia98, and

Georgia13 specific qRT-PCR assays. Common to samples from all bird types, most viruses detected were of vaccine origin, indicating that vaccines are circulating long after administration. Samples from layers and breeders taken after vaccination also showed that field administration of IBV vaccines is generally poor, which may lead to the continual circulation mentioned previously. Finally, clear profiles emerged between samples from broilers and layers/breeders, with broilers rarely having unidentified IBV and layers/breeders often having samples with an unidentified IBV by qRT-PCR. With this knowledge, it is clearly important to closely monitor vaccination application and takes to ensure proper vaccination, as well as monitor circulating field viruses even if no clinical disease is present to better understand the IBV load in an area.

The Costs of Controlling DMV/1639: A Comparison of Vaccination Strategies and Their Effects on Broiler Livability, Vaccine Clearance, and Performance

Blayne Mozisek
Merck Animal Health

The likely origin of infectious bronchitis virus serotype, DMV/1639, was in commercial layers in Pennsylvania in 2011. Within a relatively short period, the virus was affecting broilers in the Delmarva region, where it induced classic nephropathogenic disease, often confined to specific sections within a house. The severe flushing, ensuing wet litter, and stunted dirty birds that resulted became synonymous with DMV/1639. Ten years later, DMV/1639 is still an active threat to the commercial poultry industry; however, the clinical presentation has drastically changed, and its effects are now of consequence to producers throughout the major poultry-producing regions of the United States. The modern-day DMV/1639 presents more stealthy than its severe nephropathogenic ancestor. Today, clinical signs of DMV/1639 vary predominantly by region, bird size, and vaccine program, but losses are now more often associated with mild airsaculitis, reduced performance, increased culls, and/or elevated mortality. Uncontrolled, these clinical signs become more severe as the viral load in the field increases. As with all novel variant IBV serotypes, no commercially homologous vaccines are available to protect flocks from clinical disease. With rare exceptions, this leaves producers a single vaccination option – commercially available vaccines of heterologous serotypes, either alone or in combination.Lohr (1988) first discussed the protectotype concept and its importance in controlling IBV infections. The work of Jane Cook et al. (1999) then showed that the administration of Ma5 (a Massachusetts serotype IBV) at day-of-age was of critical importance to the protectotype strategy, where it provided excellent protection among many heterologous challenge viruses she examined. This case report will discuss field examples utilizing a Ma5 vaccination strategy to control the clinical signs and decreased broiler performance associated with a DMV/1639 challenge. Field studies comparing the performance of Massachusetts and GA08 serotype vaccines will also be discussed.

Evaluating the Effects of IBV Maternal Antibodies on the Development of False Layer Syndrome

Adrea Mueller
University of Georgia

Infectious Bronchitis virus (IBV) is an avian coronavirus that primarily causes respiratory disease but can also affect the reproductive tract of laying type chickens. If infection occurs in young pullets, False Layer Syndrome can develop. False Layer Syndrome is characterized by changes in oviduct development and the formation of large, fluid-filled cysts. Vaccination is used to control disease caused by IBV, but False Layer Syndrome is still seen in vaccinated

hens. We hypothesize that the presence of maternal antibodies, when combined with vaccination, will offer the most protection against cystic oviduct formation as a result of DMV/1639 challenge at three days of age. To test this, four groups of 30 SPF pullets and four groups of 30 Commercial Layer pullets were each were placed into separate colony houses at day of hatch. For each bird type, there was a negative control group, a group that only received DMV challenge, a group that was only vaccinated with live attenuated Mass vaccine oculonasally at day of hatch, and a group that received both Mass vaccine at hatch and DMV challenge. Serum samples, choanal cleft swabs, and cloacal swabs were collected at day of hatch and 5, 10, 15, and 20 days post challenge, and necropsies were performed at 20 and 30 weeks of age to collect oviducts for histological analysis. Data shows that maternal antibodies were detected at high levels in the commercial chicks at day of hatch, and all chicks were positive for vaccine in the vaccinated group and for challenge virus in all challenged groups. The data collected in this experiment will provide an understanding of how IBV maternal antibodies influence the development of cystic oviduct and False Layer Syndrome.

Vaccine Interaction and Protection Elicited by IBV Maternal Antibodies Against Early Challenge with DMV/1639

Rachel Jude
University of California, Davis

Infectious bronchitis virus (IBV) is a gammacoronavirus mainly causing upper respiratory disease in chickens. However, IBV tissue tropism can be diverse, with some strains causing urogenital issues such as nephritis and false layer syndrome (FLS). Here, we investigate the effects of maternal antibodies (mAbs) on the efficacy of a GA/08 vaccination at the hatchery, as well as the protection mAbs provide against an early challenge with DMV/1639, an IBV strain known to induce FLS. On day of hatch, specific pathogen-free (SPF) and commercial chicks were vaccinated with GA/08 and, at three days of age, the birds were challenged with DMV/1639. Samples (blood, choanal cleft and cloacal swabs) were collected at day-of-age, 5, 10, 15, and, 20 days post-challenge (DPC), and at 20 and 30 weeks of age (reproductive tissues). Respiratory signs were assessed at 5, and 20 DPC. Maternal antibodies were detected at high levels in day-of-age commercial chicks. Overall, clinical manifestations of infection were seen earlier in SPF pullets than in commercial pullets regardless of treatment (vaccination, challenge, or vaccination with challenge), indicating that mAbs may temporarily dampen, but do not preclude, clinical effects of vaccination or early challenge with IBV.

Evaluation of antibody response and virus shedding following Infectious Bronchitis Virus (IBV) vaccination with Ma5 and DMV/1639 in SPF layers

Roel Becerra
University of Georgia Poultry Diagnostic & Research Center

Infectious Bronchitis Virus (IBV) results in economic losses in layers from decreases in egg production and poor shell quality. Recently, a new variant (DMV/1639) was isolated from birds throughout the USA. To help control this variant, producers started vaccinating with a special use attenuated live DMV/1639. Because this is a new vaccine, limited data is available for antibody response and virus shedding following vaccination. In the USA, some producers vaccinate layers with a Mass type IBV at different ages to protect against the Massachusetts serotype and to serve as a “backbone” for use in a multi serotype vaccine program. There is limited data concerning antibody response and virus shedding when comparing single vs multiple vaccinations with a live vaccine. The data is even more limited when using a

DMV/1639 live vaccine. For this study, 450 SPF chicks were vaccinated on day of hatch with Ma5 via eye drop. The SPF chicks were divided into three groups and vaccinated again with Ma5 (200 birds) and/or DMV/1639 (200 birds) at 2, 6, and 12 weeks of age. A separate group of birds was kept as negative controls (50 birds not revaccinated). At 1, 3, 7, and 13 weeks of age, choanal cleft swabs were obtained from all birds for IBV qRT-PCR (Ma5 and DMV/1639 serotypes). At 2, 4, 8, and 14 weeks of age, all birds were bled for IBV ELISA and HI. This data will contribute to surveillance and diagnostic reference values which poultry companies can use when evaluating vaccination programs.

Infectious Bronchitis ELISA antibody titers as an indicator of productive impact in naturally infected broiler flocks

Jorge Chacon
Ceva Animal Health

Infectious bronchitis virus (IBV) affects respiratory and renal systems what lead to poor productive performance and increase of airsacculitis condemnation rates at the slaughterhouse. The BR-I variant strain (GI-11) is highly prevalent and has high wide distribution in South America, especially in Brazil. Normally, high ELISA antibody titers are found in broiler flocks severely affected by variant IBVs, particularly at slaughter age. A correlational study was conducted between the humoral immune response of naturally challenged flocks and the main farm and slaughterhouse parameters affected by an IBV challenge. IB antibody titers were quantified by the IDEXX ELISA kit of twenty 6-week-old broiler flocks which were detected positive for the presence of BR-I variant virus by RT-PCR. These flocks included in the study were vaccinated with Mass-type vaccine only. Productive parameters and condemnation causes of the twenty flocks were analyzed for detection of association using the Pearson correlation coefficient. As expected, flocks presenting higher mortality and airsacculitis condemnation rates had the highest ELISA titers. A positive association was found among ELISA GMT and late mortality (later than 35 days of age), mortality during transportation from the farm to slaughterhouse and airsacculitis condemnation rate. Pearson correlation coefficients indicated strong and medium linear correlation among the analyzed parameters ($p<0.05$): late mortality (0.78), transportation mortality (0.47) and airsacculitis rate (0.69). The results show that in cases of high IBV seroconversion caused by field challenges, the effect of the IBV infection on mortality and airsacculitis rates is higher.

Tracing Genomic Mutations in Spike Proteins of Infectious Bronchitis Virus (IBV) for Predictions of Neutralizing Epitopes and/or Vaccine Development

Kim Bouwman
University of Georgia

Tracing Genomic Mutations in Spike Proteins of Infectious Bronchitis Virus (IBV) for Predictions of Neutralizing Epitopes and/or Vaccine DevelopmentKim M. Bouwman, Mark Jackwood, and Brian J. JordanPoultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GAInfectious bronchitis virus (IBV) is the highly transmissible causative agent of infectious bronchitis, an economically significant disease for poultry flocks. There are many different serotypes of IBV circulating around the world due to the natural accumulation of mutations in the IBV genome during replication. When these mutations accumulate in the Spike gene, which codes for the major antigen and attachment protein, we have genetic drift and the emergence of novel serotypes. As new serotypes emerge, new live-attenuated vaccines are often produced to control disease as IBV vaccines are not broadly cross-protective. In an effort to better

understand why antibodies produced against one Spike protein may not affect the attachment of another Spike protein, sequences of the Spike protein of different circulating genotypes were obtained by direct sequencing in our laboratory or were downloaded from Genbank. Using these sequences, computational protein models were made using the published Cryo-EM structure of IBV-M41 as a reference, and amino acid changes in the genotypes in comparison to IBV-M41 were visualized. In addition, using available bioinformatic programs, predictions were made for peptides that may be important for the host immune response, and differences between genotypes in these regions were compared. A focus was placed on positions present at the surface of the protein. In addition, the post-translational N-linked glycans, which can act as a glycan shield or influence the conformation of the protein, potentially affecting ligand binding, were analysed. With these analyses we provide clues for neutralizing epitopes that can be used for vaccine development.

Assessment of mixing coccidia and infectious bronchitis live vaccines for day of age application in chickens

Robert Beckstead
Ceva Animal Health

With an increasing frequency of co-vaccination with oral coccidia vaccines and live infectious bronchitis virus (IBV) vaccines, concern has been raised regarding the potential negative effect of the 1.33 % potassium dichromate in the coccidia vaccine on IBV vaccine viability. To determine if mixing of these vaccines affects IBV vaccine titers or vaccine takes in chicks, a study was performed using optimal conditions where the coccidia vaccine was mixed into the diluent for 5 min prior to mixing in IBV vaccines and in suboptimal conditions were both the coccidia and IBV vaccines were held in contact for 3 minutes prior to mixing in the diluent for 5 min. IBV vaccine titers were determined for each treatment using Read and Muench method in embryonated SPF eggs at 0, 1, and 2 hours post vaccine mixing. No differences were observed in IBV titers between the optimal and suboptimal treatments and IBV alone at 0 and 1 hours post mixing. However, there was a 1 log reduction in IBV titers after 2 hours of contact using the suboptimal conditions. For the vaccine take portion of the experiment, coccidia/IBV vaccination in 2-day old SPF chicks was compared to IBV vaccination alone under both treatment conditions. IBV vaccine takes were determine by RT-qPCR 5 days post vaccination. There were no significant differences of Ct values between the coccidia/IBV vaccinated birds and IBV vaccinated birds under either mixing conditions. These data support the use of coccidia and IBV in a single diluent. Even though minimal consequences were detected among the different mixing scenarios at 0 and 1 hours post mixing, a safe recommendation to field veterinarians would be to follow the optimal mixing protocol.

Wealth of Knowledge

Changes to the 2021 OIE Chapter on Avian Influenza and the Economic and Trade Impacts of High Pathogenicity Avian Influenza on the U.S. Poultry Industry

Fidelis Hegngi
USDA, APHIS

Quantifying Antimicrobial Use in Poultry Production

Randall Singer
University of Minnesota

Report of the Research Priorities Committee 2022 survey results

Joel Cline
Wayne Farms LLC

The Research Priorities Committee of the AAAP conducts a survey of poultry health professionals every other year to determine where funding and efforts should be directed and allocated to address contemporary poultry health issues. In addition to conducting the survey and compiling the results, it is the duty of the committee to present the results of the survey to the industry including at the AAAP annual meeting. The Research Priorities Committee is currently developing the survey to be conducted in the winter of 2022. The results will be compiled in the spring of 2022 and this presentation will present the findings of the survey.

Does this "Stuff" Work? An Applied Poultry Researcher's Perspective on Experimental Design

Matthew Jones
Southern Poultry Research Group, Inc.

How often do you ask the question, "Does this "stuff" work?" Experimentation is the foundation for product selection, product dose, proof of efficacy in marketing, evaluating the interaction between products, and for furthering scientific understanding. While there are unlimited directions to proceed with a given project, there are characteristically fiscal and timeline constraints which influence the investigational hierarchy. The decisions made in the experimental design phase can make the difference between data that moves a project forward and data that cannot be utilized.This paper will outline the progression of research from the perspective of an applied poultry researcher. From the first steps, including product exploration, to the last steps which often revolve around application in commercial systems, each rung has a specific purpose. Every project is unique, and the sequence may differ; however, there are a few core principles that apply to both applied and basic experimentation. Biological systems are inherently variable, but in challenge models there are multiple layers of biology at play. One of the most critical steps in the planning phase is determining the appropriate number of replicates to account for biological variability. Examples will be provided to help understand how this may apply to challenge models. While planning is crucial, execution of the plan is just as important. Thus, standardization of procedures (SOPs) and training of staff ensure the integrity of the experiments. Enteric health models and food safety experiments have very different metrics used to evaluate the efficacy of products. These metrics will be explored in more detail including the best situations to evaluate these parameters. Successful experimentation is a culmination of thorough consideration and mindful execution of the protocol. This ensures integrity of the data and gives you confidence in deciding whether this "stuff" works.

Investigating true fertility of 'clear' eggs at transfer

Isabella Hannay
University of Georgia PDRC

Due to labor shortages in a post-pandemic poultry industry, broiler hatcheries are struggling to perform routine quality assessments such as breakout analysis. As a substitute, hatcheries are utilizing the "clear" number from the in-ovo injection systems to determine fertility numbers for breeder flocks.During transfer at 18-19 days, broiler hatching eggs undergo automated candling using Embrex® vaccine-saver technology to remove 'clear' eggs prior to vaccination. This project investigated whether these 'clear' eggs were truly infertile or also contained early dead embryos. The fertility of 1000 eggs, identified by an Embrex® machine as 'clears', were assessed via hand candling and breakout assessment. Fertility assessments were repeated for various broiler breeder flock ages within the following ranges: 25-30 weeks, 31-40 weeks, 41-50 weeks, 51-60 weeks and >60 weeks. This sampling

enabled comparison of the proportion of early dead and true infertile based on hen flock age and can provide a reference for expected early dead percentage. This assessment was conducted at 2 hatcheries for the same integrator where single-stage and multi-stage incubation systems are utilized to evaluate the impact of incubation system on early dead to infertile ratios. Control variables for this project included egg storage time, source flock, breed and automated candling system. The results of this project will help determine a reliable conversion factor for modern breed's true infertility from 'clears' without relying on excessive manual labor associated with egg breakouts.

Electrostatic Application of a Bound Residual Antimicrobial to Broiler Breeder Eggs: Impact on Hatchability, Chick Quality and 7-day Mortality

Mary Thompson
UGA Poultry Diagnostic & Research Center

Several studies were performed under normal broiler hatchery and field conditions to determine the benefits of Armatrex® on hatchery related performance parameters when the product was electrostatically applied to post-peak broiler breeder eggs. Armatrex® is a bound, broad-spectrum silane based quaternary ammonium antimicrobial (3-(Trimethoxysilyl) Propyldimethyl Octadecyl Ammonium Chloride) at a 1% concentration of active ingredient. In the first two studies, buggies of recently collected eggs were electrostatically sprayed with the antimicrobial compound in the farm's egg rooms with additional buggies from the same collection date remaining as non-treated (controls). In the third study, treated buggies underwent a second application following in-ovo vaccination and were placed together with control buggies in hatchers which were also electrostatically sprayed with the antimicrobial. Treated and non-treated buggies were properly labeled to track location throughout the incubation process. In each of the three studies, evaluated parameters included hatchability, early/middle/late embryo mortality, bacterial/fungal contamination, chick quality, 7-day mortality and 7 to 10-day body weights. Preliminary results revealed reductions in fungal contamination and off-odor during residue breakout analysis, reduction in bacterial and fungal growth in day-old chicks and improvement in 7-day mortality on the broiler farm.

Controlled Exposure utilizing a Hemolytic Ornithobacterium rhinotracheale Strain in Commercial Hen Turkeys to Protect Against a Field Challenge

Jessica Walters
Virginia Department of Agriculture and Consumer Services

First Week Broiler Mortality: Trends, Lesions, Etiologies and Recommendations

Jose Linares
Ceva Animal Health

First Week Broiler Mortality: Trends, Lesions, Etiologies and RecommendationsJose A. Linares, Matilde Alfonso, James Mills, Marshall PutnamCeva Animal HealthFirst week mortality is a key broiler health indicator, and it has been trending up in the U.S. broiler industry as far back as 2013. It appears to have a seasonal component with higher mortality during the colder months. In our experience, bacterial infections tend to be the most prevalent cause of mortality. Avian Pathogenic E. coli (APEC) and Enterococcus cecorum are the most common bacteria isolated. These bacteria tend to gain a foothold in the hatching egg/hatchery/broiler/broiler farm continuum. Since 2020 we have also documented other contributing factors such as viral enteritis and systemic issues (emaciation, dehydration, and visceral gout). In this presentation we'll look at trends, cases, lesions, bacteriology, APEC

Pathogenicity Gene PCR results, potential root causes and potential solutions.

Applications Used to Help Control and Eradicate Low Pathogenic Avian Influenza From Commercial Turkey Farms

Carrie Cremers
Jennie-O Turkey Store

Applications Used to Help Control and Eradicate Low Pathogenic Avian Influenza From Commercial Turkey FarmsC. M. Cremers*, M. M. Kromm*, K. M. Stumvoll*, G. Rajcic-Spasojevic**Jennie-O Turkey Store, Willmar, MN Low pathogenic avian influenza is a viral disease that causes significant economic loss for the poultry industry. In this case study, discussion will be focused on a low pathogenic avian influenza outbreak that occurred in central Minnesota in commercial turkeys. Focus of this talk will be on the different on-farm applications and management strategies that were used to help control the spread and helped to eradicate the virus. These strategies could be implemented for future low pathogenic avian influenza outbreaks to help prevent economic loss for the poultry industry.

Outreach Efforts and Newcastle Vaccine Efficacy to Promote Gamefowl Wellness in Southern California

Alejandra Figueroa
UC Davis School of Veterinary Medicine

The impact that virulent Newcastle disease has repeatedly had on backyard poultry communities in Southern California demonstrates the lack of resources and knowledge on maintaining poultry health. The Gamefowl Wellness Program at UC Davis has gained valuable input from gamefowl breeders in these affected communities on their practices, husbandry, and on the knowledge they desire. Communication channels have been preserved between gamefowl breeders and poultry specialists at UC Davis, the CA Department of Food and Agriculture, and the U.S. Department of Agriculture to continue providing resources like Newcastle vaccines, educational workshops and establishing trust. Newcastle disease vaccines have been distributed to breeders, both in person and through feed stores. To evaluate vaccine efficacy, representative blood samples have been collected from breeder flocks before and after vaccine administration. Outreach efforts as well as Newcastle vaccine efficacy in gamefowl flocks of Southern California will be discussed.

Welfare

Welfare Frameworks: History, Evolution and Practical Implementation

Katherine Weathers
Cobb-Vantress, Inc

Animal welfare can be simply defined as the relationship and synergy between the physical health of the animal and the mental well-being or behavior of the animal. To support this definition and the interconnectedness between the physical state and the mental state of animals, it is important to understand the creation and evolution of three globally recognized theories and philosophical strategies that are commonly used as the foundation framework for animal welfare standards and guidelines. These foundational frameworks include the 3 Circles Model, the 5 Freedoms of Animal Welfare and the 5 Domains of Animal Welfare. Each framework addresses the basic health, affective states, and behavior of animals and are widely used for welfare guidelines of animals utilized in food production systems, laboratories, zoos and aquariums. All three are viable frameworks that incorporate science, animal-based outcomes, and subjective human perspective when assessing welfare. However, by understanding the subtle differences with

regards to the depth of each theory, the potential for bias, and challenges with practical use, attendees can develop a broader perspective for how the frameworks can be integrated and implemented within their area of expertise.In this presentation, a brief review will be utilized to highlight the creators, origin and primary focal points of each of the foundational frameworks. Areas of overlap and differences will be mentioned as frameworks are compared and contrasted. Evolution of each framework will also be highlighted, and further details will be provided to underscore potential pros and cons for each. Examples will be provided to demonstrate subjective differences in welfare assessment that can occur with particular focus on welfare assessment of chicks in hatcheries and older chickens on farms. Finally, specific practical examples will be included to accentuate how welfare frameworks can be practically incorporated into welfare training and audits for hatcheries and farms.

The Barnyard Perspective of Animal Welfare: Learning from each other

Angela Baysinger
Merck Animal Health

This presentation will evaluate the current status of animal welfare from the perspective of the challenges and opportunities faced by food animal species. Animal welfare has a fundamental foundation for maintenance and improvement of the animals under our care. We will evaluate how the barnyard (Poultry, Swine, Cattle, Small Ruminants and Aquaculture) can collectively learn and improve the welfare of all food animals.

Cage Free Housing for Laying Hens: Floor Substrate Provided During Rearing Influences Welfare Parameters

Marisa Erasmus
Purdue University

The US is transitioning from caged to cage free housing systems for laying hens. Many cage free systems are litter-based, providing hens with behavioral opportunities. However, air quality is poorer due to increased dust and ammonia. One possible solution is to use artificial turf, but this might influence hen welfare. Hen behavior and welfare are also influenced by the rearing environment because birds develop preferences for substrates that they were exposed to early on. This study is the first in a series that examined the influence of flooring type on pullet and subsequent hen welfare. In two trials (T1: brown, T2: white pullets), we compared AstroTurf® (AT) and wood shavings (SH). Welfare parameters (footpad and feather condition, and keel bone damage) were scored for 20% of pullets (T1: 12 and 17 weeks; T2: 11 and 15 weeks). Regardless of flooring, feather damage was more prevalent at the earlier than the later timepoint in both trials, keel damage was more prevalent later on, and footpad condition of AT pullets improved over time. Flooring type has implications for pullet welfare: AT is associated with a higher incidence of feather damage, whereas SH is associated with a higher incidence of keel damage in brown birds and keel tip fractures in white birds closer to laying age. These results can guide decisions about flooring types for cage free pullets, and the implications of the rearing environment for hen welfare will be discussed further at the meeting.

Applying the Assessment of Key Indicators in Layer Chicken Systems for Improved Welfare

Joe Sullivan
Herbruck's

Understanding welfare of laying hens in different types of cage-free housing systems

Darrin Karcher
Purdue University

Cage-free housing is becoming more prominent in the table egg industry. There are three

different types of cage-free housing defined: single tier, multi-tier aviary and hybrid multi-tier aviary. Commercial cage-free facilities will be involved for data collection for the study. One hundred laying hens will be individually assessed for different welfare measures in addition to three flock level measurements. The goal of this research is to identify differences in flock and individual hen welfare in different types of cage-free housing systems.

Emphasizing Welfare Outcomes and KWIs: NCC Broiler and Broiler Breeder Welfare Guidelines Update and Audit Changes

Ashley Peterson
National Chicken Council

To assist the people and the companies who produce and process chickens for food, the National Chicken Council (NCC) developed the NCC Welfare Guidelines and Audit Checklist which has been widely adopted as a baseline by chicken farmers and processors to ensure chickens are being properly cared for and treated humanely. These guidelines cover every phase of a chicken's life and offer the most up-to-date science-based recommendations for the proper treatment and humane care of broiler chickens.The industry's approach to the well-being of broilers and broiler breeders is focused on objective measures and welfare outcomes throughout the birds' entire lives. These updated guidelines – for both broilers and broiler breeders – focuses on several Key Welfare Indicators (KWIs) which are truly measurable and indicative of broiler welfare. With the help of industry welfare leaders and poultry welfare academics, these guidelines have been updated based on this approach and the presentation will review the changes to the welfare audits as well as the rationale behind these changes.

The Case of the Missing Intestines: Peck-Outs in Broiler Breeders

Christina Lindsey
Aviagen

An increase in hen mortality in several genetic lines of grandparent broiler breeders prompted an investigation to determine the cause. Peck-outs leading to cannibalism was quickly diagnosed in the affected flocks, and a collaborative effort ensued to further characterize and address the contributing factors. This presentation will explain the veterinary, husbandry, and nutritional factors involved in the development and resolution of cannibalism in grandparent broiler breeders.

Broiler Chicken Welfare: Overview of Key Welfare Indicators Used in Broiler Production Systems and a Practical Approach to Achieve Continuous Improvement

Kathleen Long
Maple Leaf Foods

Broiler welfare is recognized as a key component of broiler production systems. With increasing interest from customers and the general public, welfare programs and outcomes are frequently audited to ensure compliance with supply chain expectations. While this focus and emphasis on welfare has resulted in a substantial number of company, customer and certification scheme audits to measure broiler welfare, there has been no standardized list or methodology for the collection of key welfare indicators (KWIs) for broiler production systems. Recently, a collaborative effort by poultry value chain stakeholders was led by the International Poultry Welfare Alliance (IPWA) to provide outcome-based KWIs for the broiler sector. These KWIs can be effectively implemented at hatcheries, farms, during bird handling procedures and transport, and at the broiler processing plant. The comprehensive guidance developed by IPWA allows for improved understanding of what outcome-based measurements should be assessed, where each KWI should be evaluated,

why the KWI is important, and how the KWI can be accurately and objectively be measured. In this presentation, broiler KWIs will be described for each segment of the broiler supply chain. Examples will be included to illustrate how KWIs can be incorporated into welfare training for company employees and farmers, internal audits, and part of welfare tracking as part of a robust welfare program for a broiler company. Additional insight will be provided in the form of a case study to describe how the use of these standardized KWIs has enhanced broiler welfare understanding and assessment, how focus on specific KWIs has driven continuous improvement in welfare outcomes, and how welfare progress is being communicated to internal and external stakeholders.

The Importance of Key Welfare Indicators in Driving Continuous Improvement in Animal Welfare Outcomes

Ken Opengart
Tyson Foods

In any system, the ability to measure outcomes to assess system performance, identify opportunities for continuous improvement, establish and execute plans for advancement, and drive accountability and engagement through communication is afforded by the use of key performance indicators. Relative to the welfare of animals within production environments, these metrics are known as key welfare indicators (KWIs). KWIs are outcome-based metrics, including animal-based outcomes, that are assessed as an indicator of positive and negative welfare outcomes. For poultry, KWIs can be measured at the hatchery or farm, during or after transport, and up to the point of slaughter. Having standard descriptions and methodologies for the collection of KWIs is critical to ensure that good quality information is collected so accurate reporting and appropriate action can be taken to address identified opportunities for improvement. A comprehensive KWI monitoring program is an important component of a robust animal welfare program as it can be used to drive continuous improvement in poultry welfare throughout the production system. The establishment of a KWI program demonstrates active engagement in the management of animal welfare which is a growing expectation of value chain stakeholders. Transparent communication of KWI progress also builds credibility and confidence with internal and external stakeholders.

Effects of novel Pulsed Alternating Wavelength System (PAWS) on welfare and skeletal quality of laying hens

Brittney Emmert
Purdue University

Proper lighting is critical for the proper development and reproduction of poultry. However, little is known on the effects of lighting type on skeletal quality and welfare of laying hens. Pulsed Alternating Wavelength System (PAWS) is a novel lighting technology with claims to improve hen welfare and productivity. As the layer industry transitions to cage-free housing, the benefits of PAWS have great potential for improving the welfare of cage-free hens. The impacts of PAWS on welfare and skeletal quality will need to be understood to use the technology in cage-free housing. This project followed commercial flocks housed in conventional cages under either conventional lighting or PAWS throughout the lay cycle. A physical welfare assessment was performed at each time point and bones from the wings, legs, and the keel were collected for analysis of bone quality parameters. This research aims to determine the impacts of PAWS on the welfare and skeletal quality of laying hens housed in conventional cages. The obtained information will be important for the implementation of the technology in the layer industry and potentially improving welfare for cage-free hens.

Understanding Outcomes-Based Welfare Assessments and Internal Audits in Turkey Production Environments

Molly Parker
Butterball, LLC

Turkey is an important part of several cultures and a source of lean protein found in many diets. But public concern for farm animal welfare has increased and what we know about raising birds well has expanded. Farmers and poultry veterinarians have worked hard to improve livability and general health in their flocks, but there is room for improvement. As with other species of poultry, outcomes-based welfare measures are the best practice for assessing, and eventually improving, turkey welfare. Beyond understanding these measures and implementing them, there must be a verification stage to ensure the protocols set are realized. This presentation will focus on verifying welfare measures are in place and being collected properly through internal audits. Standards and supplier protocols are audited against with a pass/fail mentality or a score. Key welfare indicators can be included in this process for greater overall welfare. KWIs for turkeys include measures collected at the hatchery, the farm, after transport and at slaughter. All members of the supply chain should be committed to collecting data that can be analyzed at every step of the process to address and prevent welfare challenges. The aim of this talk will be to share a case study of an internal audit process modified to include key outcomes-based welfare measures (IPWA Key Welfare Indicators Reference Guide) with insight into the special considerations needed for turkey welfare.

Potential Welfare Issues Affecting Turkey Husbandry and Well-Being

Eric Gonder
Consultant

Turkey production is distinct from other poultry in several husbandry areas that can affect turkey well-being and welfare. Some of these practices are historic and may require evaluation under modern conditions. Others are market-driven or a result of economic conditions. These run the gamut from genetics through breeding, hatching, production, transport and processing. Some can be addressed in a matter of months; others will require years or decades, necessitating careful economic evaluation and attention to development of market and industry conditions driven by retailers and society in general. Another difficulty is the disagreement between various turkey welfare programs on the same welfare parameter (i.e density, enrichments) , although each claims to be science based. Additional research is needed to determine which in fact provide improved welfare conditions for turkeys and those who care for them.

Evaluation of Foot Pads throughout Production in Commercial Turkeys

Kabel Robbins
Butterball, LLC

Foot pad quality is an important component of the American Humane Certified Animal Welfare Standards Audit Tool for Turkeys. Commercial turkey flocks were evaluated for foot pad scores prior to transfer from the brooder house and then after transfer to finisher housing and at processing. This data was used to compare foot pad scores and assess the relative importance of litter quality and management at different phases of production. Geography, density, bedding substrate, ventilation type and other parameters were compared to foot pad scores as well to try and better understand potential contributing factors to foot pad quality with a goal of continuous improvement.

“Good as Hell”: A Discussion Regarding Toe Treatments of Hen Poults

Katie Stumvoll
Jennie-O Turkey Store

As animal welfare and animal rights become more of a mainstream issue, focus will continue to increase on physical treatments applied to commercial poultry. Taking a proactive approach, Jennie-O Turkey Store is researching alternatives and modifications to the current model of toe treatment on commercial hen poults. Nutrition, density and pain mitigation will be discussed.

A Comparison of Various Euthanasia Devices and Methods in Turkey Hens

Brian Wooming
Cargill Turkey & Cooked Meats

This presentation will review the results of three different studies that examined the efficacy of different devices at achieving immediate, irreversible euthanasia on turkeys. Each device will be also be evaluated on the criteria of durability, ease of use, and cost for on-farm use.Study one looked at a Carbon Dioxide (CO2) system for euthanasia of 13 and 33 day old turkey hens, using a 20 second CO2 prefill and 120 second cycle. Video recordings for each euthanasia event were analyzed for behavioral indicators of distress, insensibility, and death. The behavioral indicators of distress were head shaking and gasping. The behavioral indicator of insensibility was loss of posture. The behavioral indicators of death were cessation of rhythmic breathing, cessation of movement, and defecation.Study two compared several captive bolt devices (Turkey Euthanasia Device, Zephyr-EXL, Jarvis Stunner, Experimental Crossbow), mechanical cervical dislocation (Broomstick method [BRM] and Koechner Euthanasia Device [KED]), and manual cervical dislocation (MAN) methods on 8 and 12-week-old turkey hens. Each method was assessed for impact on loss of brain stem reflexes, euthanasia success, and torn skin. The cervical dislocation techniques were also analyzed via radiograph for proper dislocation. Furthermore, each device was assessed for physical parameters. Study three looked at a manual non-penetrating crossbow on eleven and twenty week old turkeys. Euthanasia effectiveness was measured by observing reflexes of brainstem insensibility including: jaw tone, eye nictitating membrane response to touch, eye pupillary constriction response to light, the occurrence and duration of gasping and end time of convulsions, wing flapping duration, and absence of sustained breathing. Post-mortem, body weight was recorded and scores were collected for external hemorrhage/skin laceration (0-2 scale), subcutaneous hemorrhage (0-4 scale), and skull fracture (0-3 scale).

Welfare Communication Tools for Field Veterinarians

Nancy Reimers
Cutler Associates

Maintaining well-being in flocks requires regular communication with stakeholders including caretakers, certifiers, owners, and consumers. Each group uses distinct vocabulary and responds best to a targeted approach. Practitioners who tailor communication enhance education for those who make daily impacts on bird health and management. AAAP/AVMA/PSA provide resources to help field veterinarians negotiate effective education for the betterment of bird health and welfare.

Enteric Health

Comparison of Antimicrobial Susceptibility Testing of Clostridium perfringens by the Agar Dilution and the Broth Microdilution Methods

Martine Boulianne
Université de Montréal

Antimicrobials are commonly used in the poultry industry for the prevention and treatment of

bacterial infections. However, the use of antibiotics in food animal production has been associated with the emergence of drug resistance in pathogens. The Clinical and Laboratory Standards Institute (CLSI) suggests following the agar dilution method when testing for antimicrobial susceptibility for Clostridium perfringens, the causative agent of necrotic enteritis. However, multiple studies on C. perfringens are using the microdilution method, which is faster. In this study, the minimal inhibitory concentrations values of 64 field isolates of C. perfringens were obtained by the standard agar dilution method and by the microdilution method, and values were then compared. Due to high variability in the values obtained for most of the antimicrobials by the microdilution method, we were not able to calculate correlation coefficient between both antimicrobial susceptibility techniques. This study highlights the importance of following CLSI protocols when testing for antimicrobial sensitivity for C. perfringens.

Natural Prevention of Coccidiosis and Necrotic Enteritis in Experimentally Infected Broilers and Analysis of Gene Expression in Intestinal Tissue Samples

Felipe Mendy
BIOVET S.A.

Coccidiosis and necrotic enteritis (NE) are major poultry diseases caused by Eimeria and Clostridium perfringens (CP), respectively. Pronutrients (PN) and Cimenol Ring (CR) are active molecules from plants used to control these enteric infections. PN are intended to prevent coccidiosis by reinforcing the gut immune system and CR is an antimicrobial highly effective against CP.A trial was conducted to evaluate PN and CR as preventive tools for coccidiosis and NE in broilers. Treatments were an uninfected untreated control (NC) fed a diet without anticoccidials/antimicrobials; an untreated Eimeria-infected group (PC1); an untreated Eimeria and CP infected group (PC2). These groups were supplemented either with PN alone or with PN and CR together, which made a total of five supplemented groups: NC+PN, NC+PN+CR, PC1+PN, PC2+PN, and PC2+PN+CR. P0.05); and in challenged groups compared to PC1 and PC2 ($P<0.05$). PN alone significantly improved FCR compared to PC1; however, the improvement was numerical compared to PC2.PN up-regulate immunomodulatory genes that take part of the host protective immunity against Eimeria.In summary, PN are highly effective against coccidiosis, though the combination with CR is a better option when coccidiosis is complicated with NE. Besides, PN increase the expression of immune-protective genes in the mucosa. PN and CR are natural, do not leave residues, nor create resistances.

Effect of A Feed Sanitizer on The Control of Necrotic Enteritis in Broilers

Enrique Montiel
Anitox Corporation

Effect of A Feed Sanitizer on The Control of Necrotic Enteritis in BroilersEnrique MontielAnitox1055 Progress Circle, Lawrenceville, GA 30043, USAemontiel@anitox.comThe effect of a feed sanitizer in a necrotic enteritis (NE) challenge model was evaluated. One thousand four hundred and forty broilers were vaccinated with a coccidiosis vaccine and divided into 5 groups of 288 chickens each and housed in pens on litter, 24 birds per pen. A commercially available feed sanitizer was used in the feed of groups 1n 2 and 3 at an inclusion rate of 6 pounds per ton and treated feed was offered to birds as follows: group1, day 1 to day 17; group 2 from day 1 to day 35 and group 3 from day 17 to day 35. Group 4 and 5 received untreated feed to serve as negative and challenge controls, respectively. At day 17, all birds in groups 1,2,3 and 5 were challenged with the field Clostridium perfringens strain CL-15 via feed. Lesion scoring was

assessed at day 21 using a scale of 1-4. Duodenum, jejunum and ceca samples for histopathology were collected at 21 and 35 days. Oocyst count in feces were conducted at 21 days of age. Specific NE and total mortality were recorded from day 1-35 and body weights and feed intake used to calculate feed conversion and weight gains at days 21, 28 and 35. Lesion score, specific NE and total mortality after challenge were significantly lower in the 3 treated groups as compared to the untreated-unchallenged group. Average weights, weight gain and feed conversion were also improved in the treated groups. All results will be discussed.

Experiences using a Recombinant Attenuated Salmonella Vaccine to control Necrotic Enteritis in Commercial Broilers

Andrea Zedek
Huvepharma

Necrotic enteritis (NE) is a severe enteric disease of chickens, caused by toxins produced by the bacteria Clostridium perfringens. The high mortality and production loss manifested by this enterotoxemia is extremely costly to the poultry industry. A newly licensed Recombinant Attenuated Salmonella Vaccine (RASV) protects against NE by expressing Clostridium perfringens genes coding for a-toxin & NetB toxin, which stimulates vaccinated birds to produce antibiodies against these toxins. Throughout 2021, paired house trials and larger production trials were performed in commercial broiler facilities to evaluate the zootechnical performance of this vaccine. Results will be discussed.

Beneficial effects of tea extract and cinnamon oil on avian coccidiosis

Inkyung Park
USDA-ARS

Two experiments were conducted to evaluate the effects of tea extract (TE) and cinnamon oil (CO) on intestinal health and necrotic enteritis. In experiment 1, an in vitro culture system was used to investigate the individual effects of TE and CO on the proinflammatory cytokine response of chicken macrophage cells (CMC), gut integrity of chicken intestinal epithelial cells (IEC), and differentiation of quail muscle cells and primary chicken embryonic muscle cells. In experiment 2, in vivo trials were carried out to study the effect of a combination of phytochemicals used in experiment 1 on coccidiosis in broiler chickens infected with E. maxima. One hundred male broiler chickens (0-day-old) were allocated into the following five treatment groups: (1) Control group without infection (CON), (2) Basal diet with E. maxima (PC), (3) Phytochemical diets (T1:10, T2:20, and T3:40 mg/kg feed) with E. maxima. Body weights (BW) were measured on days 0, 7, 14, 20, and 22. Jejunal cytokines and TJPs were measured at eight days post-infection (dpi). Fecal samples for oocyst shedding were collected from 6 to 8 dpi. In vitro, phytochemical treatment reduced IL-1ß and IL-8 from LPS-activated CMC, enhanced gene expression of TJ and MUC-2 of IEC. There was no killing activity against Eimeria tenella sporozoites or Clostridium perfringens bacteria, even at high doses. In vivo, phytochemical treated chickens showed enhanced BW, reduced oocyst shedding, and decreased IL-1ß following E. maxima challenge. In conclusion, the dietary combination of TE and CO demonstrated the beneficial effects on intestinal protection, immune responses, and growth in broiler chickens challenged with coccidiosis. Therefore, the present finding provides scientific rationale to develop phytochemical feed additives to enhance intestinal health in broiler chickens.

The impact of Salmonella Typhimurium and coccidiosis vaccine on microbiome and intestinal integrity markers in broiler chickens

Andrea Pietruska
Auburn University

Over the last years antibiotic-free broiler production in the US has increased to 58%. To reduce the risk of salmonellosis for consumers and to reduce economic losses by coccidiosis, antibiotic-free and organic productions have increased the use of vaccines against both pathogens. Previous research indicated that Salmonella Typhimurium infections in broilers were more severe after vaccination against coccidiosis. Additionally, the expression of tight junction genes and cecal microbiome showed significant changes after administration of both vaccines. In this study, we used a 2 x 3 experimental design with different vaccination schedules on day 0 and 14 of broiler chick age to further investigate the interactions between vaccines against coccidia and S. Typhimurium. The six groups consisted of six floor pens each with 45 broiler chickens per pen. On day 0 the chicks were tested to confirm that they were negative for vertically transmitted Salmonella. Environmental samples were taken every week to detect the Salmonella vaccine strain. Samples of jejunal and cecal content, blood for plasma, and cecal wall were taken on day 28 pi. Gene expression analysis by qPCR was performed to investigate expression of tight junction genes in the ceca. A microbiome analysis of ceca and jejunum was performed by Illumina sequencing of 16S rRNA. In addition, the expression of systemic and cecal antibodies against S. Typhimurium was investigated. Samples from the last timepoints are currently being evaluated.

Differential Analysis of Jejunal mRNA of Broiler Chickens Challenged with Eimeria maxima with or without Clostridium perfringens

Nima Emami
University of Georgia

Subclinical necrotic enteritis (NE) is responsible for the greatest economic impact on poultry production. However, research models can lead to clinical NE with significant mortality, which often does not reflect field conditions. Thus, we compared Clostridium perfringens (Cp) strains #4 and #6 in an infection model with Eimeria maxima (EM) to induce a subclinical NE. A total of 33, day (d)-old broilers were individually wing-tagged and raised till d 14 when they were allocated to one of four treatment groups: 1) non-challenged control (n=4); 2) EM (n=9); 3) EM+Cp#4 (Cp4; n=10); and 4) EM+Cp#6 (Cp6; n=10). Birds were orally gavaged with 1 mL of 3,000 EM oocysts on d 14. Birds in Cp4 and Cp6 groups were orally gavaged with 1 mL of Cp cultures on each of d 19 and d 20 (~1×108 CFU/mL). Birds were individually weighed on d 14, 19, 20, and 21. On d 21, jejunum samples were collected to measure mRNA abundance of interleukin (IL)-1?, IL-6, IL-10, C-C motif chemokine ligand (CCL)-4, CCL5 and CCL20, CARD domain containing (NLRC)-3 and NLRC5 inflammasome, and leucine-rich repeat and pyrin domain containing (NLRP)-3 inflammasome. Data were analyzed using JMP (Pro16) and significance (P?0.05) between treatments were determined by LSD test. Cp6 had greater mRNA abundance of IL-1? and IL-6 compared to NC and EM, while Cp4 did not affect these responses. The impact of Cp4 and Cp6 on mRNA abundance of chemokines and inflammasomes are currently being evaluated. Based on these preliminary results, Cp#6 appears to exert greater influence on mRNA abundance of immune response genes in jejunal tissues of commercial broilers than both EM and Cp#4.

Identification of arthropod vectors of Histomonas meleagridis and Heterakis gallinarum in broiler breeder farms

Maria Tereza Bethonico Terra
Auburn University

Histomonas meleagridis is commonly found in broiler breeder pullet farms. It can use the cecal worm Heterakis gallinarum as a vector and reservoir. Moreover, cecal worm eggs are not

only very resistant in the environment but can also be carried by arthropods. However, little is known about which arthropod species are the most robust vectors. The aim of this study is to define relevant arthropod vectors of H. meleagridis and H. gallinarum in broiler breeder flocks.Over a period of one year, four broiler breeder pullet farms were sampled every four months. On each farm, three types of traps were set inside and outside two houses and remained for a period of one week. Trapped specimens were identified at the order or family level morphologically. Selected specimens were tested for the parasites by PCR. Preliminary results showed a higher abundance and total number of arthropods outside poultry houses than inside. Eleven different orders were identified and, besides darkling beetles, 644 individuals were counted. Diptera was the most popular order with Sphaeroceride, Sciaridae and Musciduae being the most prevalent families. PCR analysis showed some samples that were positive for only Heterakis or Histomonas and others positive for both.Samples from the last timepoints are currently being evaluated and next step include identification of potentially vectors of these two parasites by PCR. This study can provide knowledge to designing targeted programs against specific vectors to reduce prevalence of both parasites and transmission between flocks.

Focal Duodenal Necrosis: identification of Gram-negative rod-shaped bacteria in intestinal lesions

Yu-Yang Tsai
The University of Georgia

Focal Duodenal Necrosis (FDN), an intestinal disease is among the top five disease concerns in table egg layers. In this research, we aimed to identify the Gram-negative rod-shaped bacteria commonly found in FDN lesions. A total of 59 ethanol-fixed duodenum samples were collected from 8 FDN-affected farms and 42 samples had typical FDN lesions. Macroscopically, there were focal to multifocal superficial erosions in the duodenal mucosa. Microscopically, heterophilic and lymphoplasmacytic enteritis with mild enterocyte necrosis at the villous tips, mild to moderate luminal fibrinonecrotic exudate, and variable numbers of long rod-shaped bacteria were observed within lesions. Laser Capture Microdissection followed by sequencing of the 16S rRNA gene were performed to identify the bacteria found within lesions. Sequencing analysis revealed Proteobacteria as the predominant phylum in FDN-affected samples. Pseudomonas spp., Pseudomonas veronii, and Enterococcus faecium were the top three bacteria detected. Ten duodenal samples with FDN lesions were collected for bacteriology and a total of 47 colonies grew on MacConkey and blood agar plates. Through 16S rRNA gene PCR and Sanger sequencing, 39/47 colonies were identified as Escherichia coli. PCR for E. coli virulence genes revealed avian pathogenic E. coli (APEC) virulence genes in 70.2% of isolates and 93.6% of isolates contained Inflammatory Bowel Disease virulence genes. FDN appears to be a multifactorial inflammatory intestinal disease associated with dysbiosis and the proliferation of Gram-negative bacteria that may contribute with the pathogenesis of this disease.

An Update on Turkey Viral Enteritis Diagnostic Cases in Commercial Turkey Poults in California

Shayne Ramsubeik
California Animal Health and Food Safety Laboratories Turlock, UC Davis

Poult enteritis complex is a significant health problem for the intensive turkey industry globally, resulting in considerable economic losses. The condition is often times multifactorial and enteric viruses have been detected in the intestine of both healthy and poor performing poults. Recently, molecular detection of enteric viruses became available for routine diagnostic

use at the California Animal Health and Food Safety (CAHFS) Laboratories offering a more sensitive and specific alternative for virus detection and identification. Analysis of poult necropsy submissions for the period February 1st 2020 to February 1st 2021 revealed 65.1% (43/66) of submissions diagnosed with turkey viral enteritis. Diagnosis was based on correlation between viral detection, clinical presentation and gross and microscopic findings. In addition, the highest frequency of detection occurring within the first week of life The most common macroscopic findings included pale, thin-walled, fluid-filled small intestines; thin, dilated, fluid-filled ceca and primarily litter in the gizzard. The most common microscopic findings included villi changes (atrophy, fusion, blunting), infiltration of the lamina propria with mixed inflammatory cells and hyperplasia of crypt epithelial cells.

Surveillance and diagnosis of turkey coronavirus using environmental bootie swabs

Becky Tilley
Butterball LLC

Turkey coronavirus (TCV) is a highly contagious enteric disease of turkeys. Clinical signs of TCV can range from grossly normal to severe diarrhea, morbidity, and mortality. Control during outbreaks of TCV requires quarantine of positive farms and controlled marketing or depopulation. Surveillance and diagnosis of TCV rely on TCV PCR and serology. This presentation will discuss use of environmental bootie swabs for TCV PCR testing during a large outbreak of TCV and correlation of these results with clinical signs and TCV serology.

Salmonella

Understanding NPIP's Pullorum-Typhoid Clean Programs

Elena Behnke
USDA National Poultry Improvement Plan

The National Poultry Improvement Plan (NPIP) is a cooperative federal-state-industry program for the application of new diagnostic technology. The foundation for the NPIP resides within Pullorum-Typhoid Clean programs. Recently, there has been renewed interest in comprehensively reviewing the strengths of the PT Clean programs, including the historical context and basis for serologic testing, past outbreaks of Pullorum and their economic impacts to the poultry industry, numbers of birds tested for qualification and maintenance, trading partner acceptance of the programs, and other benefits of participant enrollment. The General Conference Committee, an elected official advisory committee to the United States Secretary of Agriculture, was asked in the Fall of 2021 to collaborate with the USDA NPIP to appoint a Pullorum-Typhoid Working Group, in order to address a myriad of questions and concerns that have resulted from the past couple of years of struggle with supply availability of antigen utilized for the plate agglutination test. Though the previous antigen shortage is no longer problematic, the importance of maintaining steady supply of plate antigen and having adequate diagnostic support for these PT Clean surveillance programs cannot be underscored enough. This presentation will focus on specific research findings from the PT Working Group, including highlights from 2022 Biennial Conference.

Genetic characterization of Salmonella Infantis recovered from comminuted turkey samples collected by the USDA Food Safety and Inspection Service between 2019 and 2021

Roxana Sanchez-Ingunza
RSI Poultry Veterinary Consulting LLC

In 2016, Salmonella performance standards were established for two types of turkey

products in the United States (U.S.), turkey carcasses and comminuted turkey. The maximum acceptable percent positive in comminuted turkey for the poultry establishments to meet the standard is 13.5%, while the prevalence of Salmonella in this type of regulated product was 18.34% in 2020. Salmonella enterica subsp. enterica serovar Infantis (S. Infantis) is within the top five most important Salmonella serovars recovered from turkey samples as reported by the FSIS. S. Infantis emergent clone reported in the U.S. carries several antimicrobial resistance genes and other genes, providing the bacteria with environmental survival advantages. Previously, we have described plasmid-associated genetic variation in S. Infantis recovered from chickens in South America and genetic variability in U.S. chicken isolates linked to plasmid content and chromosomal mutations. The present study investigates the genetic features of S. Infantis recovered from comminuted turkey in the U.S. between 2019 and 2021. The genome sequencing data of a total of one hundred S. Infantis isolates from comminuted turkey collected by the FSIS were downloaded from the NCBI website. The isolates were genetically characterized through single nucleotide polymorphism (SNP) phylogeny analysis, plasmid detection, antimicrobial profiles prediction, gene content evaluation, and detection of gene mutations leading to truncated proteins. This study aids in the understanding of S. Infantis circulating in turkey processing establishments, the identification of molecular targets for diagnostics and process control, and in defining control interventions in the field and at the processing plant.

Impact of a New Vaccination Approach in Pullets and Broiler Breeder Layers to Reduce Salmonella Contamination in Broilers.

Jaime Ruiz
Elanco Animal Health

Impact of a New Vaccination Approach in Pullets and Broiler Breeder Layers to Reduce Salmonella Contamination in Broilers.2022 AAAP meeting - Philadelphia July 29 - August 2 Jaime Ruiz, DVM, MSc, MAM, Diplomate ACPV; Lynn Warren, Ph D; Sandra Aehle, MSc; and Brandon Carter, MSc Veterinary EpidemiologyContamination of retail poultry products with Salmonella has important public health implications. With increasing pressure from regulators and consumers to guarantee safe poultry products, the poultry industry must continue to develop control strategies aimed at reducing Salmonella infections in pre-harvest production. Risk management must include a plan of comprehensive standard practices, including pre-harvest interventions, such as administration of vaccines, to reduce and control Salmonella infections in poultry and environmental contamination. Vaccination of poultry against Salmonella infections is a complementary intervention in an overall Salmonella control program. Vaccination against Salmonella infections aims to mimic the development of naturally acquired immunity in poultry. Live and killed vaccines, when used together in pullets, have been shown to reduce vertical and horizontal transmission of Salmonella in meat birds (Young et al 2007, Dorea et al 2010). By raising the resistance to Salmonella infections through vaccination of breeders, Dorea et al (2010) showed that the Salmonella burden on broilers at slaughter was significantly reduced. A trial was conducted to evaluate the impact of a combination of live and inactivated (Salmonella enteritidis SE) Salmonella vaccines in pullets and broiler breeders during lay. Vaccination during the laying period included live Salmonella vaccine boosts for the life of the flock beginning at week 40. The effect of the new vaccination approach in the following variables will be presented: 1.- Breeder BioCheck ST/SE ELISA serology before and after the new vaccination program. 2.-

Salmonella incidence in hatch tray swabs, boot swabs for broiler, pullets and hen farms. 3.- Carcass rinses - Plant Salmonella testing (hot rehang, pre chill, post chill and parts), and, 4.- Pullet livability at capitalization before and after the new vaccination program.Dorea, F., D.J. Cole, C. Hofacre, K. Zamperini, D. Mathis, M.P. Doyle, M.D. Lee and J.J. Maurer. 2010. Effect of Salmonella vaccination of chicken breeders on reducing carcass contamination of broiler chickens in integrated poultry operations. Appl. Environ. Microbiol. 76:7820-7825.Young, S. D., O. Olusanya, K. H. Jones, T. Liu, K. A. Liljebjelke, and C. L. Hofacre. 2007. Salmonella incidence in broilers from breeders vaccinated with live and killed Salmonella. J. Appl. Poult. Res. 16:521-528.Elanco and the diagonal bar logo are trademarks of Elanco or its affiliates. © 2021 Elanco and its affiliates. PM-US-21-3247

Comparison of Whole Cell Bacteria and SRP Salmonella Vaccines in Broiler Chickens: Evaluation of Protection Against Homologous and Heterologous Salmonella Strains

G Ritter
Poultry Business Solutions LLC

Comparison of whole cell bacteria and SRP Salmonella vaccines in broiler chickens: Evaluation of protection against homologous and heterologous Salmonella strainsG. Donald Ritter, DVM, ACPVPoultry Business Solutions LLC, Norfolk, VAMilos Markis, Ph.D.AviServe LLC, Newark, DE 19711Salmonella control is one of the top priorities for poultry producers due to the potential of the bacteria to infect humans and cause severe disease. Salmonella in poultry is typically controlled through biosecurity and vaccination. Serotypes of Salmonella have been identified based on somatic O-antigens, and generally these antigens do not confer cross-protective immunity when used to manufacture inactivated whole cell bacteria vaccines. Siderophore receptors and Porins (SRPs) are highly conserved pore proteins on the surface of gram-negative bacteria, including Salmonella, that transport essential nutrients and iron required for bacterial survival. Unlike serotype-specific antibodies produced against Salmonella whole cell bacteria, antibodies targeted against Salmonella SRP proteins have been shown to be cross-protective against multiple Salmonella serotypes. Vaccines utilizing SRP proteins or inactivated whole cell bacteria from Salmonella Enteritidis or Salmonella Infantis were compared for protection against homologous and heterologous challenge in broiler chickens with Salmonella Enteritidis or Salmonella Infantis. These two strains were chosen for this comparison because they do not share any somatic O antigens. Salmonella clearance from spleens following intravenous challenge was used to evaluate prevention of organ invasion. Intestinal shedding of Salmonella was evaluated after oral challenge. Findings from these studies will be presented.

Case Reports

Outbreak Of Multiple Subtypes Of Low-Pathogenic Avian Influenza In Waterfowl And Gamebird Flocks

Carmen Jerry
UC Davis

Low pathogenic avian influenza was diagnosed in a flock of adult commercially raised quail. The birds had an initial complaint on the premise of drops in egg production and watery droppings. Initial testing of the flock for avian influenza was negative, however, mortality started increasing drastically, which prompted further examination and the submission of more birds to the diagnostic laboratory. On ante-mortem examination, the birds appeared lethargic with ruffled feathers and had labored breathing. Gross examination revealed birds that were in poor body condition, with moderate dehydration and splenomegaly, ovarian

regression, and airsacculitis was seen. Several hens had thin shelled and shell less eggs. Microscopically, lymphoplasmacytic encephalitis with neuronal cell necrosis, fibrinoheterophilic pneumonia, splenic lymphoid depletion with amyloid deposition was seen. The influenza virus was characterized as low pathogenic H7N3. A flock of waterfowl, in close location to the quail was found positive for H5N2 serologically, these birds were asymptomatic. This premise had a previous history of low and highly pathogenic avian influenza of the subtypes H5N2 in quail.

Case Report: Investigating Unusual Outbreaks of Neurologic Disease in Broiler Chickens

Natalie Armour
Mississippi State University

A broiler integrator in the southeastern United States experienced outbreaks of neurologic disease in 4-week-old broiler chickens on three farms (A, B, and C) of one complex in the spring and summer of 2018. Affected birds were submitted in March (farms A and B) and July (farm C) for necropsy. A majority of birds in each case displayed severe central nervous system (CNS) signs, including torticollis and head tremors. Several birds were also markedly lame, with splayed legs. Gross lesions included swelling of the gastrocnemius and digital flexor tendons, cerebral and cerebellar congestion, and bursal atrophy. Histologically, there was mild to severe lymphocytic perivascular encephalitis, lymphonodular tenosynovitis, and lymphocytic infiltrates in multiple organs. PCR testing was performed to rule out Avian Influenza, Newcastle Disease, Avian Encephalomyelitis and Marek's Disease, while negative culture results excluded bacterial and fungal etiologies. Avian Reovirus was isolated from all tendon and brain pools following passage in chicken embryo liver cells. Based on amino acid sequences of the sigma C product, the reoviruses from farms A and B were determined to be in Genotype 4, while those from farm C were identified as Genotype 5, variant group 1. Results of in-situ hybridization (ISH) using riboprobes specific for the isolated viruses to detect mRNA in fixed tissues will be presented. While these cases represent only the second reported instance of reoviruses associated with CNS signs in chickens in the United States, they suggest that reovirus infection should be considered as a differential for neurologic disease in chickens.

My Birds Seemed Terrified!

Martha Pulido Landinez
Mississippi State University

In April 2021, two cases from small flocks of MS laying hens were received for necropsy at the MSU – PRDL. Both submitters reported a history of sudden death. According to the history, the birds showed normal behavior until a few hours before death. No clinical signs or behavior changes were identified previously. Dead hens were found mainly inside the nests or in the corners of the coop. All birds had a history of regular food intake and water consumption. Egg production was as expected. According to the information provided by one of the submitters, not all the hens in the pen died, but the survivors looked utterly terrified. The most obvious problem identified during carcass evaluation was a severe external and internal buffalo gnats/black fly infestation. A preliminary entomological analysis identified these flies as Simulium meridonale, the turkey gnat, one of the most common black fly species in Mississippi. Macroscopic and microscopic lesions suggested these hens suffered a massive attack while inside their nesting boxes or perhaps tried to hide there. Although in most of the cases of black fly attacks reported in the literature, the death is attributed to the toxemic effect of multiple bites. Analysis of these two cases suggested that these hens also suffered severe suffocation due to numerous flies blocking the upper respiratory system, especially blocking the choana and trachea. Implications of

these results in the differential diagnosis of acute mortality in hens will be presented, as well as an apparent increase in black fly attacks on backyard poultry from these avian pests.

Cleaning up an Outbreak

Laura Tensa
Cargill

The Shenandoah Valley is a densely populated poultry production area located in western Virginia, comprising of intermixed broiler, layer, breeder, and turkey farms. There are approximately 670 broiler farms and 265 turkey farms in the valley. Historically, this area has been infected with both turkey corona virus and avian influenza in the late 1990's and early 2000's, and rapid transmission was present throughout the industry resulting in depopulation.TCV, also known as turkey coronavirus, or bluecomb, is a viral enteric disease. Clinical signs include backing off feed and severe flushing leading to unevenness and stunting in flocks. Drastic impacts are noted at processing with underweight flocks with a high feed conversion and low daily gains in both hens and toms. PCR and serology are the tests offered at select diagnostic labs.In November 2020, a case of turkey coronavirus was diagnosed in the Shenandoah Valley from a flock with clinical signs. As tested increased, it quickly became apparent that the virus had already begun spreading rapidly throughout the valley. In total, 54 farms across seven counties and two states were diagnosed, until the last flock processed April 2021. In order to successfully contain the outbreak, all turkey companies with birds in affected areas coordinated testing, processing, movement, cleaning, disinfecting, and repopulation of control zones. This case study will outline two production companies experiences in successfully controlling the virus in different production systems, and lessons learned and applied going forward.

Histomonas meleagridis outbreak & transmission pathway investigation on a private farm in Tennessee, United States

Richard Gerhold
University of Tennessee College of Veterinary Medicine

The University of Tennessee Center for Veterinary Medicine's Molecular Parasitology lab was contacted by a veterinary student because of a possible Histomonas meleagridis (Blackhead) case on a family's small private farm in Oak Ridge, Tennessee (USA). On the farm, chickens and turkeys were housed in adjacent pens and allowed to graze in the same area. A turkey with clinical presentation of lethargy and sulfur-colored feces was observed and died seven days after initial clinical signs. Necropsy performed on the turkey revealed severe liver target lesions consistent with blackhead. Histological analysis confirmed the diagnosis of blackhead. Liver and cecal cultures inoculate into Dwyer's media and Hollander's fluid revealed various flagellated protozoa. Following the initial mortality, four of five remaining poults on the farm died with the same clinical signs and gross findings upon necropsy. Culture of the liver and cecum of these carcasses revealed similar organisms as seen in the initial bird. To confirm the transmission cycle from chicken to turkeys, soil from the farm was sampled and processed to collect any Heterakis gallinarum eggs to determine if the eggs contained H. meleagridis DNA. The H. meleagridis sequences from soil samples, if detected, will be compared to the sequences from the liver and cecum of poults with blackhead. In addition to confirming the presence of H. meleagridis in culture, Tetrarrichomonas gallinarum and Simpliconomas spp. were also present in culture.

A Pestilential Poult Problem

Jacob Carlson
Select Genetics

In early July, a brooder barn containing 5-day old turkey breeder poults began experiencing significant mortality. Initially, antibiotic treatment was attempted based on clinical signs, microscopic findings on gut scrapings and gross necropsy. After multiple days on antibiotics with no decrease in mortality or clinical signs, formalin fixed tissue samples and whole bird specimens were collected and sent to the University of Missouri Veterinary Medical Diagnostic Laboratory to rule out causes of young poult mortality. Microscopically, numerous cryptosporidia were detected embedded in the brush border of the epithelial linings of the villi in the ileums. At about 28 days of age the affected flock finally achieved acceptable levels of mortality and were no longer clinical for cryptosporidia infection.

Case report: Clinical investigation of a turkey barn with a recurrent history of histomoniasis

Vijay Durairaj
Huvepharma Inc

Histomoniasis causes devastating losses to the turkey industry. Recurrent histomoniasis outbreaks have been consecutively documented in a Midwest turkey barn. The first outbreak occurred in Fall 2018 and continuous outbreaks were documented in the subsequent placements. The tenth histomoniasis outbreak was reported on July 2021 in 9500 six-week-old turkeys. An extensive clinical investigation was conducted to confirm histomoniasis and to identify the possible factors involved in histomoniasis transmission. Dull and depressed birds were observed upon clinical investigation. Several samples were collected from the affected turkeys and farm premise. Fibrinonecrotic cecal cores and hepatic necrosis were observed on necropsy. Microscopic evaluation revealed necrotizing heterophilic typhilitis and hepatitis with abundant infiltration of Histomonas trophozoites. Histomonas meleagridis was isolated and identified as genotype-1 by gene targeted sequencing. Houseflies collected at the farm tested positive for H. meleagridis. This case report will elaborate on the gross and histologic lesions associated with histomoniasis, isolation and identification of the pathogen, and potential vector involved in disease transmission.

Increased Cull Eggs in an Upper Midwest Turkey Breeder Operation

Benjamin Wileman
Select Genetics

This case report describes the findings of a case of increased cull eggs in a small division of breeder turkeys. This presentation will also cover a small bone ash survey results from this operation as well as cover a flock supplementation trial that was done to investigate the efficacy of various supplementation strategies.

Investigation into an Outbreak of Turkey Coronavirus and Histomonas Infections on Several Turkey Farms

Brian Wooming
Cargill Protein

This presentation will describe the investigation into dual infections of Turkey Coronavirus and Histomonas in commercial turkey flocks. These flocks were grown on 26 farms located in a small community in central Missouri. The farms are in a poultry dense location that also has numerous, small, pasture-raised, organic layers. Affected turkey flocks performed poorly with decreased livability, low body weight, and increased feed conversion at processing.Turkeys were diagnosed with Turkey Coronavirus and Histomoniasis in April 2020. The area was depopulated, as this was perceived to be the most reliable method to eradicate the problem. Repopulation was starting in February 2021, and positive cases of both diseases were again detected by July 2021. Interestingly, the layers in

the vicinity also tested positive for both Turkey Coronavirus and Heterakis gallinarum, the common vector for Histomonas meleagridis, This case study will discuss chickens as a potential reservoir for both diseases, and the supporting laboratory data will be presented.

Investigating urolithiasis in broiler breeders

Randi Clark
Sanderson Farms

Avian gout occurs secondary to renal damage induced by various primary agents. The most common reported causes include dehydration, excessive dietary calcium and infectious bronchitis. Commercial layers that receive layer rations too early in rearing have been reported to experience visceral and renal gout due to the formation of uroliths made up of calcium sodium urate leading to ureter blockage and ultimate kidney failure. Two of four broiler breeder pullet houses in a complex in south Georgia lost approximately 20 birds a day to gout beginning at 14 weeks of age. Mortality continued in the hen house and was not impacted by feed acidification, the proven treatment in layers. Urolith analysis showed the stones were composed of sodium acid urate monohydrate. Feed deliveries and bronchitis titers provided additional information but no clear origin of the stones or explanation of their composition has been identified.

Broiler breeder pullets: wrong diet, high mortality, unusual lesions!

Tahseen Abdul-Aziz
Rollins Animal Disease Diagnostic Laboratory

Clinical history: 20-day-old broiler breeder pullets were presented to a veterinary diagnostic Laboratory for necropsy. The pullets were from house #3 on a broiler breeder pullet farm with four houses. 13,000 pullets and 3,500 males separated by a partition were placed in the house. The mortality in the pullet's pen from day 10 through 19 are as follows: 45, 40, 44, 110, 129, 119, 210, 259, 261, and 350. Pullets in the flock were small for their age. Clinical signs have not been noted on birds in the flock. The mortality was "normal" in the male's section in house #3, in houses #1 and #2 (13-week-old pullets), and house #4 (20-day-old pullets). Toxicosis was suspected. Gross lesions: In most of the birds, the proventriculus was markedly enlarged and has a thickened wall, with multifocal to coalescing, moderate reddening of the mucosal surface. There were superficial erosions and irregular thickening of the koilin layer of the gizzard of some birds. In some birds, the kidneys were mildly-to-moderately-to-markedly enlarged, with accumulation of urate in the ureters. Frothy material was present in the thoracic and abdominal air sacs of a few birds. Tissues were collected for histopathology. Histopathologic lesions were found in different organs and tissues. Histopathologic lesions were present in the proventriculus, liver, kidneys, air sacs, and gizzard.

Case Report: Kinda Wish it was the M Word!

Sara Throne
Simmons Foods Inc

Coryza is an infectious disease caused by the bacterium Avibacterium paragallinarum. This case report will describe multiple cases of Coryza in breeder flocks ranging in age from 40 to 51 weeks of age involving more than 58,000 birds. The case initially presented as sudden mortality in a two-house farm. The birds experienced sudden increase in mortality accompanied by respiratory symptoms consisting of severe dyspnea, swollen sinuses, purulent exudate and severe morbidly. Initial rule outs consisted of Mycoplasma gallisepticum (MG), Mycoplasma synovia (MS), and Avian Influenza (AI). By the time these tests were returned with negative results, three (3) more farms were identified as having clinical signs. Further work up with additional samples, expanded the rule out list to

include Cholera and Coryza as primary rule-outs, along with many other less probable causes. Avibacterium was able to be cultured from several samples and was confirmed via PCR and WGS in multiple labs. Discussion of the treatment and response to treatment will be discussed. Upon epidemiological investigation, the bacterium was introduced into these four (4) flocks via asymptomatic spike males. The spike males were transferred from a flock of 25-week-old breeders. The primary flock never exhibited clinical signs and continued in production. No means of introduction into the source flock was ever identified.

Case Report: HPAI in a Maryland Broiler Farm

Michael Quist
University of Georgia, PDRC

What Are Your Chickens Drinking?

Kurt Dobson
George's Inc.

This is a case of knowing what your birds are eating and drinking. An eight house farm that in house one at 7 days of age the birds had backed off of feed and water. Then in all houses around the age of 23 days went through a period of severe flushing and increased mortality. With some increase of water and then backing off. It is critical to look at water source and mineral analysis prior to growing birds in the house.

Case Report of Lessons Learned with Mycoplasma Synoviae Surveillance

Philip Stayer
Sanderson Farms

National Poultry Improvement Plan (NPIP) mycoplasma clean status requires routine flock surveillance for the pathogen. NPIP approves serological samples of the flock taken at designated times to assure no flock exposure to mycoplasma. This real-life case report illustrates a clinical presentation of Mycoplasma synoviae (MS) in commercial broiler breeders that was disseminated to several farms before discovery even though originating flock sera samples taken in accordance with NPIP were negative at the time of dissemination. Epidemiology of this MS break demonstrates the limitations of mycoplasma serology.

Epidemiologic Considerations from an HPAI outbreak in Dubois County Indiana

Duane Murphy
Farbest Farms

On February 6, 2022 a commercial turkey farm (identified as D1) in Dubois County, Indiana broke with H5N1 avian influenza (HPAI), apparently acquired from wild waterfowl in the area that were also diagnosed with H5N1 HPAI. Over the next 3 weeks, 3 additional commercial turkey farms (D2, D3, and D4), all within a 2.5-mile radius, broke sequentially with H5N1, each farm breaking 7-8 days after the farm before it. Because of the timing and close proximity, horizontal transmission between these farms was suspected. Genetic vSNP analysis at the National Veterinary Services Laboratories revealed a pattern of stepwise changes in these 4 viral isolates that were consistent with direct or indirect transmission between the 4 premises, supporting the hypothesis of horizontal transmission, though the outcomes of phylogenetic analysis should only be interpreted in context of all available virus and epidemiologic information and should not be used alone to infer transmission. Other than geographic proximity, there were no obvious epidemiologic links between these 4 farms. Possible routes of farm-to-farm transmission will be explored, and implications for depopulation and farm management procedures during an outbreak will be considered.

A peculiar Avibacterium paragallinarum Infection in Layers with Complete Absence of any Clinical Presentation of Infectious Coryza

Amro Hashish
Iowa State University

Infectious coryza (IC) is an important respiratory disorder of chickens caused by Avibacterium paragallinarum (AP). Recently, there has been increased incidence of infectious coryza in the United States. In May 2020, a submission of 6 pools of "Choanal swabs (OP)" was received for Realtime PCR (qPCR) of AP. The choanal swabs were taken from layer flocks no clinical signs. Unexpectedly, the results were positive. Amplification of the hypervariable region of HMTp210 gene was attempted; however, all positive qPCR samples revealed negative results.Thirty sentinel birds were placed within the positive flocks. Fourteen days later, the sentinel birds were submitted for necropsy examination, bacterial isolation and molecular detection. Moreover, metagenomics whole-genome shotgun sequencing was also executed. The sentinel birds were normal during the necropsy examination. OP swabs from sentinel birds showed positive results using qPCR. Metagenomics data analysis revealed the presence of a considerable number of AP reads. Additionally, two AP were successfully isolated. AP identity for both was confirmed using MALDI-TOF. Bacterial whole-genome sequencing (WGS) was done for further genomic characterization of both isolates. However, WGS analysis revealed significant differences between both isolates and other available AP references genomes. The absence of some virulence factors (e.g. absence of one locus within the fimbrial gene) and meaningful differences within the important major hemagglutinin antigen HMTp210 gene were identified. Further confirmation of lack of clinical signs should be proved by a challenge study using the isolated AP.

Pathology

West Nile Virus Infection in a Developer Pekin Duck Flock

Richard Fulton
Michigan State University

A flock of six-week-old Pekin developer ducks, selected to be breeders, was experiencing an increase in daily mortality. Six birds were submitted dead for necropsy. All of the birds had hydropericardium. Four of the six birds had white streaks on the epicardial surface of the left and right ventricles. One bird had a diffusely pale left ventricle. Three of the birds had ascites. Heart and brain were positive by PCR and immunohistochemistry for West Nile virus. Additional PCR testing for Eastern Equine Encephalitis, avian influenza, and Newcastle viruses was negative. Microscopically there was a necrotizing myocarditis and a cerebral vasculitis. Myocarditis is a common feature of West Nile virus infection in other bird species.

Reoccurrence of West Nile Virus Infection in Pekin Breeder Ducks Associated with a Drop in Egg Production

Mayra Tsoi
Michigan State University

A sudden drop in egg production in commercial bird flocks can be economically devastating and rapid identification of the underlying cause often requires a combined effort between the producer, veterinarian, and avian pathologist. In September 2021, three Pekin breeder duck flocks aged 32, 58, and 62 weeks from three farms in Indiana experienced a 41.2% drop in egg production and increased mortality. Seventeen hens and one drake including live and dead animals from each flock were submitted to Michigan State University Veterinary Diagnostic Laboratory for further investigation. Gross necropsy findings affecting multiple birds included: flaccid/shrunken ova (11), atrophied ovary (6), pododermatitis (6), and hepatomegaly (4). Histopathologic examination of cerebrum and cerebellum revealed multifocal mild perivascular cuffing by lymphocytes, endothelial

cell hypertrophy, and small glial nodules, which was highly suspect for a viral encephalitis. PCR for Newcastle disease, avian influenza, eastern equine encephalitis, and West Nile virus (WNV) was performed. Brain samples were positive for WNV by PCR and WNV antigen was detected in the molecular layer of the cerebellum by immunohistochemistry. Depending on the avian species infected with WNV, there may be no gross or histologic findings in highly susceptible species (such as crows) that die acutely, or encephalitis, endophthalmitis, and myocarditis in susceptible species (such as bald eagles). In our case, infection of Pekin ducks with WNV presenting as a drop in egg production is an unusual manifestation of this disease, and represents the second occurrence in Indiana since 2019.

An unusual case of systemic Histomoniasis in a backyard turkey poult

Emily Pittman
Georgia Poultry Laboratory Network

One 4-month-old turkey poult was submitted to the Georgia Poultry Laboratory network for diagnostic necropsy after being found dead in the pen by the owner. According to the owner, the other four birds in the pen exhibited mild depression and lethargy with mustard-yellow feces. Necropsy showed classic lesions of Histomoniasis, including cecal cores and multifocal pale regions in the liver of varying degrees. In addition, on the kidneys there were multifocal round lesions with irregular edges, some with a dark red center and pale periphery. There were also pale foci of various sizes throughout the pancreas. Histologically, there was massive necrosis in the liver. Multifocal granulomatous inflammation with intralesional protozoa, consistent with Histomonas meleagridids, were noted in all four organs affected grossly.

Black Livers at Processing in Broiler Chickens

Veronica Nguyen
Department of Population Health and Reproduction, UC Davis School of Veterinary Medicine

Two 6- to 7-week-old broiler chickens were recently condemned and examined post-slaughter. Intriguingly, both had uniformly dark livers but no other gross abnormalities. We investigated whether protoporphyrin accumulation was associated with our dark liver samples and explored other potential causes of the dark liver phenotype.Formalin-fixed and fresh frozen liver samples were obtained and examined via optic and electron microscopy (EM). Through EM, we detected crystals composed of needle-like structures that were refractile when exposed to polarized light.To determine whether protoporphyrin was the major and/or sole cause of the dark liver phenotype, we looked at possible contributions from pathogens, metal toxicities, and liver deposits. Liver samples were also explored through histological staining with Perl's Iron Stain and Hall's Bile Stain, to detect hemosiderosis or bile, respectively.Overall, our results confirm that protoporphyrin accumulation is associated with the uniformly dark liver phenotype. As protoporphyrin is converted to heme via ferrochelatase, the accumulation of protoporphyrin is suggestive of either insufficient quantities or activity of ferrochelatase. This underscores that detection of this phenotype is not just a gross incidental finding but an indication of heme synthesis dysfunction. Further reporting and recognition of future cases are crucial in establishing a pattern among affected broiler chickens.

Spontaneous Testicular Teratoma and Facial Xanthomas in a Spitzhauben Rooster

Jarra Jagne
Cornell University Animal Health Diagnostic Center

Spontaneous Testicular Teratoma and Facial Xanthomas in a Spitzhauben RoosterJarra F. Jagne, Andrew Miller and Carmen R. SmithIthaca, NY 14850A one year-old Chamois Appenzeller Spitzhauben rooster was presented to the New York State Animal Health Diagnostic Center with a history of dyspnea,and large growths with surface dark scabs on the upper and lower eyelids of the left eye. A referring veterinarian observed yellow raised lesions in the trachea and a large abdominal mass by ultrasound,Necropsy revealed a very large mass occupying approximately 60% of the coelomic cavity. It was 14 x 7.5 x 7cm, multilobular to coalescing,soft, heterogeneously red, yellow, tan, and black and greasy, with hundreds of white, hard- and thin-walled structures filled with clear, thin, fluid distributed diffusely throughout the parenchyma and appears to originate from the left testicle. The mass was diffusely covered by yellow, friable material (fibrin) and dozens of randomly distributed, up to 2.0 x 1.0 x 2.5 cm, thin- and transparent-walled structures with yellow, translucent fluid (cyst). The coelomic and pericardial cavities contained approximately 10 mL and 0.5 mL, respectively, transparent, thin fluid.Histological examination further revealed a large, unencapsulated, multilocular mass composed of abundant cysts with variable morphology. Many of the cystic spaces are lined by elongate, spindle-shaped cells with bright eosinophilic cytoplasm. These spaces often contain a transition from a reticulated network of polygonal cells with abundant non-staining cytoplasm to condensed, ovoid nuclei stippling a prominent amphophilic matrix (chondrogenesis; cartilage). Several of these cystic structures focally contain anastomosing trabeculae of bright eosinophilic matrix that are stippled with condensed nuclei and larger, polygonal cells with non-staining cytoplasm (bone).Teratomas are considered uncommon neoplasms in poultry. This presentation will describe teratomas in poultry in addition to the specific case report.

Coccidiosis

Using Eimeria Lesion Scores and Fecal Oocyst Counts in Addition to Final Body Weight as Parameters in Floor Pen Studies

Rüediger Hauck
Auburn University

For decades, the most important means to mitigate the consequences of infections with coccidia in broiler production was the use of anticoccidials. However, fewer or even none of the traditionally used feed additives can be used in antibiotic-free and organic production. This situation has caused a search for alternatives like pre- and probiotics or herbal extracts. Often these experimental products are tested in floor pen studies, which simulate field conditions including the cycling of coccidia. The success of any treatment can be measured with a variety of parameters. The two most important economic metrics, which are closely related, are body weight/ body weight gain and feed conversion. Lesion scores and oocyst shedding are more directly correlated to replication of the parasites in the intestinal tract. However, these two parameters are not necessarily correlated with broiler performance as measured by weight gain and feed conversion at processing.A meta-analysis of experiments done in floor pens was performed using all three parameters as metrics of the efficacy of traditional anticoccidials and alternative feed additives like herbal products, pre- or pro-biotics. The hypotheses were that there was a higher correlation between the three parameters in birds treated with more potent, traditional anticoccidials than in birds treated with alternative feed additives and that traditional anticoccidials were more effective. A total of 44 experiments from 43 articles in peer reviewed journals were identified to fit the

inclusion criteria and relevant data were extracted. The data are currently analyzed.

OPGs: What do they mean?

Hector Cervantes
The University of Georgia

Oocysts per gram (OPGs) have been used for many years to evaluate effectiveness of anticoccidials and development of immunity in poultry, however, interpretation of OPGs is not easy, many factors must be considered when interpreting OPG results. The following factors can have a significant effect on the number of oocysts detected: 1) type of coccidiosis prevention program in use, i.e. anticoccidial drugs (chemicals vs. ionophores) vs. vaccines (attenuated vs. non-attenuated), 2) age of the flock when the samples are collected, 3) initial level of contamination, 4) type and amount of sample collected (i.e. droppings vs. litter), 5) downtime period between placement of flocks, 6) number of samples/house and the location from where those samples were collected, 7) immune status of the bird, 8) crowding effect, 9) predominant Eimeria spp. present in the sample and its pathogenicity, 10) storage conditions and time of storage prior to oocyst counting, and others. Under experimental conditions oocyst counts are much more useful because the influence from most of the variables listed above plus the many uncontrolled variables that exist in the field has been minimized. As more poultry is produced under NAE, RWA, and organic programs, the use of synthetic anticoccidials and vaccines has increased. Development of resistance to synthetic anticoccidials can be problematic and OPGs could be used as an early warning sign. OPGs could also be used to monitor proper cycling of coccidial oocysts and development of active immunity in birds vaccinated with live coccidiosis vaccines.

Validation a Portable Cell Counter for Enumeration of Eimeria species of Chickens

Rocio Crespo
North Carolina State University

Rapid non-invasive diagnostic tools for coccidia (Eimeria spp.) quantification and speciation, that can be performed on-site are both challenging and necessary. Recently, we demonstrated that flow cytometry can be used to enumerate and speciate coccidia oocysts with high accuracy. However, its application at the barn side is challenging. Hence, we utilized the Image J software (https://imagej.net/Welcome) for scientific image analysis that can be efficiently coupled with McMaster slide method. We soon realized this system was impractical for use at the farm. Automated cell counters, that are used for cellular work, can analyze many samples in a short time. These instruments consist of a digital camera and the analyses are performed through specialized software that requires a minimal user involvement. These units can be easily transported to the farms. For this study, we validated a protocol for quantification of Eimeria sp. in poultry, using a cell counter. We used two automatic cell counters, Luna II and Countess 3, to enumerate oocysts from commercial vaccines. The counts were compared to McMaster preparations. Each sample was evaluated in triplicate. Linearity and range of the automatic and manual methods were evaluated using solutions prepared at the same concentrations and percentages. There was a significant positive correlation between the manual McMaster method and the automated cell counter. However, crystals in the saturated salt solution resulted in an overestimation (constant bias) of oocyst. Although further validation is needed, the results from the automatic cell counters are encouraging that would allow fast sample processing and objective results.

Evaluation of Litter Moisture Analyzers and the Effects of Litter Moisture on Cycling of Coccidiosis Vaccine

Nicholas Brown
Huvepharma Inc

High litter moisture has been associated with increased severity of coccidiosis as well as prolonged oocyst survival. However, litter moisture can be difficult to objectively quantify on the farm due to the requirement for oven-drying of samples to obtain an accurate result. In addition, the precise moisture levels needed to promote or inhibit coccidial cycling are not well known. The purpose of this study was two-fold: 1) Assess the accuracy of handheld analyzers and the Adams Equipment PMB 202 device in measuring litter moisture as compared to the gold standard traditional oven-drying method, and 2) to assess if measured differences in litter moisture cause any detectable changes in vaccine cycling during a 5 week grow-out. To accomplish this, broiler chicks were inoculated with coccidiosis vaccine and reared for 5 weeks in floor pens maintained to have two distinct moisture levels (<20%, and ~30-40%). To assess coccidial cycling, lesion scoring was conducted throughout the trial. Litter samples were taken at several timepoints to determine which devices correlate best to the traditional oven method. The results of this study are pending.

Analyzing Nanopore NGS Data of Vaccine and Field Isolate Eimeria for Identification

Benjamin Jackwood
UGA

Correct identification of Eimeria parasite species is crucial for disease control as no cross immunity exists between species. Vaccinating birds typically protects them from disease, however oocysts of vaccine origin persisting in the environment after use can be a drawback of using vaccine. Using microscopic species differentiation makes it challenging to know if an oocyst identified in a sample field is a true coccidiosis challenge or simply a remnant from previous vaccination. Nanopore next generation sequencing (NGS) technology is a novel approach to identifying Eimeria parasites and can provide more detail for species differentiation. From our previous work, performing NGS on amplicons of identifying gene regions produced high quality profiles from sequence data which identified Eimeria strains in commercial vaccines. NGS data from three different genome regions, Internal Transcribed Spacer-1 (ITS1), Ribosomal 18S DNA (18S), and Cytochrome Oxidase 1 (CO1), was used to differentiate species of coccidia. Amplicons of each gene were generated from four commercial US coccidiosis vaccines and sequenced in triplicate by nanopore NGS. Field isolates from chicken farms, both where vaccination occurred and where the farm had no history of coccidiosis vaccination, were used to produce amplicons for nanopore NGS. Sequence data displays nucleotide differences in 18S and ITS1 with field vs vaccine, but also shows that CO1 alone, while still being able to differentiate species, may not be enough to classify origin. These results demonstrate the applicability of using nanopore sequencing technology for differentiating Eimeria species in mixed samples, and allows insight to which identifying gene regions may be used for origin classification.

In Ovo Vaccination of Chickens Against Eimeria Maxima Infection Using Recombinant EmaxIMP1 Protein Linked to Nanoparticles

Mark Jenkins
ARS-USDA

In ovo vaccination of chickens against Eimeria maxima infection using recombinant EmaxIMP1 protein linked to nanoparticlesMark C. JenkinsAnimal Parasitic Diseases Laboratory, ARS, USDA, Beltsville, MD 20705Previous work in our laboratory found that oral inoculation of broiler chicks with recombinant EmaxIMP1 protein linked to nanoparticles (NP) provided excellent protection against Eimeria maxima infection. With the goal of developing a practical

subunit vaccine against coccidiosis, embryonated chicken eggs (HR708) were injected with NP-EmaxIMP1 or NP-NR control protein at 18-19 days incubation either in the amnion or in the air cell. A subset of in ovo-inoculated chicks were necropsied 6 hr after hatch; spleen, bursa, and a 2 cm segment of the small intestine flanking the yolk sac diverticulum were removed and frozen to prepare thin sections for epifluorescence microscopy. Chicks were either challenged with Eimeria maxima oocysts at 14 d or 28 d post-hatch. The former evaluated by micro-oocyst counts/PCR, the latter by weight gain and FCR. Amnion injection, but not air cell injection, lead to localization of EmaxIMP1 to spleen, and small intestine. Chicks challenged at 14 days after amnion immunization with EmaxIMP1 displayed lower intestinal oocyst counts and PCR intensity than chicks immunized with NR protein. Similarly, EMaxIMP1-immunized chicks challenged at 28 d displayed excellent protection against weight gain depression associated with Eimeria maxima challenge. However, this protection was dependent on exposure to low level (1-5 oocysts) Eimeria maxima booster. These data suggest that in ovo injection with NP-EMaxIMP1 may represent a practical way to prevent coccidiosis in broiler chicks, but that protection requires exposure to low levels of Eimeria maxima oocysts as commonly observed in litter. Studies are underway to test IMP1 homologues in Eimeria acervulina and Eimeria tenella for similar immune-protective effects against coccidiosis infection.

Control of Poultry Coccidiosis by Vaccination in Pigmented Broilers in Mexico

Francisco Rios-Cambre
MSD Salud Animal Mexico

Generation of resistance to anticoccidial drugs has forced the poultry industry to look for effective alternatives for controlling coccidiosis, such as the use of vaccines. In Mexico, skin pigmentation in broilers is very important. One of the main factors that affect pigment absorption in the gut is both clinical and subclinical coccidiosis. Aiming at evaluating the implantation of vaccinal strains of Eimeria, and its effect on the absorption of pigment, we performed a full diagnostic monitoring in coccidia vaccinated broilers in the field. One-day-old chicks were vaccinated with a commercially available vaccine by spray at the hatchery. Feces samples were collected every 7 days for oocyst quantification. Postings were performed and intestinal samples were taken from 5 birds at 6, 7, 15, 16, 21, 22, 23, 28, 35 and 42 days for measuring lesion scores and for histopathological analysis. Blood samples were taken 21, 35 and 42 days for pigment detection in blood plasma by measuring its absorbance in an ELISA reader. Oocyst counts per gram of feces at different ages showed the implanting of the different Eimeria species contained in the vaccine. Pigment levels in blood plasma showed that the use of this vaccine did not affect the absorption of pigment significantly.

Everything Comes Down to Poo - Most Effective Form of Vaccination Application Using a Commercially Available Live Coccidia Vaccine

Jolene Tourville
Jennie-O Turkey Store

Coccidiosis can cause primary disease in turkeys as well as result in secondary complications. Poor production results can be attributed to uncontrolled coccidiosis. In commercial turkey production there is limited coccidiostat availability as well as potential for resistance. An alternative to using a coccidiostat is vaccination. Because proper vaccination depends on application technique it is important to assess which application process is best. Different methods of oral application were evaluated for vaccination success. Flocks were vaccinated either on farm or at the hatchery via coarse

spray. A subset of farms was also revaccinated one week post initial vaccination to assess if re-exposure improves control of coccidiosis. Fecal samples were collected at specific timepoints and evaluated for oocysts. Other parameters measured include mortality, weights, livability, and feed conversion.

Comparison of Broiler Performance Between Various Coccidia Vaccination Programs Using a Novel Rapid Oocyst-Per-Gram Enumeration Method

Andrew Bishop
Amick Farms

Comparison of broiler performance between various coccidia vaccination programs using a novel rapid oocyst-per-gram enumeration methodAndrew Bishopa, Bill Rickena, Marie Severyna, James Bartonba Amick Farms, LLC, 2079 Batesburg Hwy, Batesburg-Leesville, SC, 29006. b Ancera, 15 Commercial Street, Branford, CT, 06405The control of coccidiosis was critical for the development of the modern, vertically integrated poultry industry. The linkage of feed conversion to control of coccidia has been demonstrated to the point that it has become dogma. However, practicing corporate veterinarians still operate with limited understanding of the dynamics of coccidia cycling, as it relates to vaccination, the development of immunity, and the emergence of resistance. This limited understanding does not reflect poor training as it relates to the coccidian life cycles, but rather a paucity of available data, due to the effort involved in sample collection as well as the laboratory assessments required to determine oocyst concentrations in the feces. Because rapid and convenient quantification of the parasite life forms has not been attainable, poultry veterinarians rely on intermittent assessments through necropsy as well as the lagging indicator of flock settlements.Due to the recent availability of an automated method for quantifying oocysts per gram (OPG) in broiler feces, a project was undertaken to measure OPG at four ages (7, 21, 28, and Processing) across nine different anticoccidial vaccination plans, multiple manufacturing serials, two complexes and seventy farms. Patterns of OPG and bird positivity for oocysts will be presented, as will comparisons between economic performance of the different groups. This preliminary work introduces an automated system for quantifying OPG which can function as an early indicator of flock performance and identify new areas for research regarding anticoccidial vaccine application and the development of immunity.

Effect of a Blend of Essential Oils on Growth Performance and Intestinal Lesions during a mixed Eimeria Challenge or Vaccination in Two Studies

Sharon Heins Miller
Devenish Nutrition LLC

Commercial poultry production is moving away from prophylactic use of antibiotics. Numerous studies over the last 10 years established the benefits of essential oils. Our objective is combining essential oils into one product (DeviSTAT) and testing against a known competitor product in a controlled facility and in the field with a coccidia vaccine. The first study performed at SPR compared product D (DeviSTAT) with an inclusion rate of 0.75 lb/US ton to product M with an inclusion rate of 1.0 lb/US ton. The birds were challenged at 14 days with a coccidia inoculate with a combination of Eimeria maxima, Eimeria acervulina, and Eimeria tenella. The trial ended on day 20. The two products had similar effects on feed conversion both being statistically better than the positive controls. Product D was able to improve coccidia lesions scores as compared to product M.The second study was performed on a commercial farm research facility. All birds received a commercial hatchery coccidia vaccine. The test birds received 0.75 lbs/US ton of product D in the

starter and grower (days 0-28) and grown to 48 days. Body weight and weight gain were similar for both groups (control and product D). Feed conversion was better for the broilers supplemented with product D from day 15 to 48 (P 0.001; 5.8-point improvement). In today's commercial poultry environment, integrators are searching for alternative solutions to disease challenges and vaccination reactions as well as solutions that bolster intestinal health.

The Impact of Coccidiosis Control Programs on the Field Performance of US Poultry Farms

Ha-Jung Roh
Merck Animal Health

Coccidiosis, an intestinal parasitic disease caused by Eimeria species, is a real economic threat to the poultry industry worldwide. Both clinical coccidiosis, with symptoms such as mortality, diarrhea, and dehydration, and subclinical coccidiosis, typically without any symptoms, are responsible for the economic loss by reducing feed efficiency in chickens. To prevent coccidiosis, two approaches are widely used in the poultry industry: 1. Anticoccidial drugs administered in a feed medication program, and 2. Live coccidia vaccines. Anticoccidial drugs can be classified as either chemical or ionophores (which are considered to be antibiotics in the U.S). As each anticoccidial drug has a different level of effectiveness toward each Eimeria spp. as well as different modes of action, wide varieties of chemical and ionophore combinations are used to improve the effectiveness of the anticoccidial treatment. Unlike the coccidiostatic or coccidiocidal effects of anticoccidial drugs, a live coccidia vaccine is a prophylactic approach that can induce long-lasting protective immunity in the host and is also considered more sustainable for long-term use. While randomized controlled studies using battery cages or floor pens are considered the gold standard for coccidiosis treatment research, they do not reflect the real-world situation. The implication of the study findings is often limited, and the lack of standardization makes the comparison of the studies difficult. To understand how different coccidia control programs affect the performance outcomes of chickens in the real world, we obtained aggregated de-identified U.S. field data from poultry farms for the period of January through December 2020. This blinded data set provided multiple performance indexes such as feed conversion ratio and flock mortality of farms with various coccidiosis control programs. Additionally, the impact of seasonal changes was examined within the different programs.

Production of chicken tumor necrosis factor-a (TNF-a) in coccidiosis and necrotic enteritis

Hyun Lillehoj
USDA

Tumor necrosis factor-a (TNF-a) is a pleiotropic cytokine associated with the regulation of systemic inflammation and host defense. TNF-a is a type II transmembrane protein with either membrane-bound or soluble forms and is a prototypical member of the TNF superfamily. Chicken TNF-a (chTNF-a) is a long-missed avian ortholog, and its immunological properties remain largely unknown compared to those of its mammalian counterparts. To facilitate our understanding of the role of chTNF-a in avian diseases, a new ELISA based on newly developed anti-chTNF-a mouse monoclonal antibodies (mAbs) was developed. Here, we report Eimeria- and Clostridium perfringens-induced TNF production as measured by this new anti-chTNF-a mAbs. Furthermore, intracellular expression of chTNF-a in primary immune cells or cell lines derived from chickens was validated by immunocytochemistry and flow cytometry assays using both 3G11 and 12G6 mAbs which neutralized chTNF-a-induced nitric oxide production in chicken HD11 cells in vitro. Collectively, our results enhance our understanding of the functional characteristics

of chTNF-a, and these anti-chTNF-a mAbs will serve as valuable immune reagents to inform on inflammatory responses and disease pathogenesis in the fundamental and applied studies of avian species.

Changes in gut microbiome, immunity, antioxidant capacity and parasite fecundity induced by Oraltreatment of transgenic Bacillus subtilis-cNK2 in E. acervulina-infected broiler chickens

Samiru Wickramasuriya
USDA-Agricultural Research Service

Our recent study (Wickramasuriya et al., 2021) reported the successful construction and delivery of a stable transgenic Bacillus subtilis carrying chicken NK lysin peptide (B. subtilis-cNK2) as an effective oral carrier of antimicrobial peptide to the gut to show its therapeutic effect against Eimeria parasites in commercial broiler chickens. This study was conducted to further investigate the effects of B. subtilis-cNK2 treatment on gut microbiota, humoral immunity, mucosal antioxidant response and parasite fecundity in coccidiosis-infected broiler chickens. One hundred broiler chickens were divided into four treatment groups in a completely randomized design: 1) negative control (NC, unchallenged), 2) positive control (PC: challenged without B. subtilis), 3) B. subtilis with empty vector (EV), and 4) B. subtilis-cNK2 (NK). All chickens were challenged with 5,000 sporulated Eimeria acervulina (E. acervulina) oocysts through oral gavage except the NC group on day 15 (0 dpi). B. subtilis (EV or NK) were given to chickens orally from day 14 to day 18 with 1×1012 cfu/mL spores. Digesta and intestinal tissue samples were collected on 6 dpi to assess the gut microbiome and antioxidant makers. Fecal samples were collected from 6 to 9 dpi to enumerate the oocyst shedding and blood samples were collected on 13 dpi to assess serum Eimeria profilin-specific antibody levels. Results showed a major shift in gut microbiome profile of E. acervulina-infected chickens compared to the uninfected control and B. subtilis-EV chickens. Interestingly, B. subtilis-cNK2 group showed a similar gut microbiome profile as the uninfected chickens. Finally, B. subtilis-cNK2 treated chickens showed reduced fecal oocyst shedding (p0.05) humoral immunity and oxidative response compared to the positive control. Therefore, this study shows many beneficial changes associated with the B. subtilis-cNK2 delivery system, showing that it is a practical and effective delivery strategy to mitigate negative effects of avian coccidiosis in commercial broilers.

Infectious Bursal Disease

Isolation and Characterization of Antigenic Variant Infectious Bursal Disease Viruses in the United States

Milos Markis
AviServe LLC

Infectious bursal disease (IBD) is a highly contagious disease of chickens. It is caused by Infectious Bursal Disease Virus (IBDV) infection. The virus' primary targets are B lymphocytes in the Bursa of Fabricius, and infections early in life of chickens can result in severe and permanent immunosuppression. Immunosuppressed chickens are unable to mount humoral immunity post-vaccination and to fend off infections by secondary adventitious agents. IBDV has world-wide distribution, but the disease has been controlled successfully via vaccination. In the United States, IBD is controlled primarily through breeder vaccination with inactivated IBDV vaccines and passage of maternal immunity to the progeny, which can prevent early IBDV infections and development of severe immunosuppression. We have isolated and characterized several antigenic variant IBDV isolates since 2016 that are not neutralized by the antibodies induced by the IBDV vaccine

strains. Antigenic, pathogenic, and genetic characterization of the variant IBDV isolates will be discussed in detail.

Evaluating The Antigenic Relatedness Of Diverse Infectious Bursal Disease Virus Strains

Vishwanatha Reddy Avalakuppa Papi Reddy
The Pirbright Institute

Infectious bursal disease virus (IBDV) is endemic worldwide, and eight genogroups have been identified based on the sequence of the hypervariable region (HVR) of the VP2 capsid. There is a need to evaluate the ability of commercial vaccines to neutralize the different genogroups, including to understand the relationship between IBDV HVR sequence diversity and antigenic diversity. To address this, we used an in-house reverse genetics system and the chicken B-cell line DT40 to rescue a panel of chimeric-IBDVs with the backbone of a lab-adapted PBG98 strain and the HVRs from diverse field strains: classical F52-70 (genogroup 1), US-variant Del-E (genogroup 2), Chinese-variant SHG19 (genogroup 2), very-virulent UK661 (genogroup 3), M04/09 distinct (genogroup 4), Italian ITA-04 (genogroup 6), and Australian-variant Vic-01/94 (genogroup 8). Groups of 6 chickens were inoculated with vaccine 228E or wild-type F52-70, serum was obtained at 28 days post-inoculation, and the neutralization titer determined against the panel. Chimeric-viruses containing the HVR from Del-E, and SHG19 were significantly less neutralized by serum of F52-70, than chimeric-viruses with the HVR from UK661, or F520-70 ($p0.05$). However, only chimeric-viruses with the HVR of Del-E and SHG19 were significantly less neutralized by serum of 228E than chimeric virus with the HVR of F52-70 ($p<0.05$). In summary, our assay can be used to evaluate the antigenic relatedness of diverse IBDV strains, including to identify the contribution of individual HVR mutations to antigenicity.

Assessment of a Variant IBDV Challenge in Chickens Utilizing Combinations of Recombinant HVT and Live Attenuated IBD Vaccines

John ElAttrache
Ceva Animal Health

SPF chickens and commercial broilers were utilized to evaluate different rHVT and live IBD vaccines when applied alone and in combination at day of age. Single and dual construct rHVT vaccines were evaluated when administered alone and in combination with a live attenuated variant IBD vaccine. Day of age SPF chickens and progeny from two commercial parent flocks, which were assessed for maternal derived antibodies (MDA), were vaccinated, and then challenged with an AL2 like variant IBDV. Assessments included vaccines takes for the rHVT vaccines, serology pre-challenge, gross lesion and histopathological bursal scoring and bursal body weight ratio analysis. Correlation of all assessments was observed for both the SPF birds and commercial broilers from a higher and lower MDA parent source. The live attenuated variant IBD vaccine performed better in SPF birds when administered alone or with a rHVT vaccine. The single construct rHVT IBD with and without the live attenuated IBD vaccine provided the best results against an AL2 like variant IBDV challenge in commercial broilers when compared to dual construct rHVT IBD and live attenuated IBD alone. No significant differences were observed in the progeny from higher and lower MDA lines.

Broiler Protection and Performance Trials of the Newest HVT-IBD Recombinant Vaccine Against AL2 and Group-6 IBDV Challenges

Kalen Cookson
Zoetis

AL2 viruses are the most prevalent IBDV type in the broiler industry, having been recovered from about half of all flocks sampled over the past

decade. Group-6 viruses, while a more antigenically diverse group of viruses than AL2, have also demonstrated an ability to override high Del-E type antibodies in progeny challenge studies. However, to date no recombinant HVT-IBD vaccine studies have been presented evaluating Group-6 protection. This paper will present broiler trial results of the newest HVT-IBD recombinant vaccine on the market. Trials will include 18-19 day AL2 and Group-6 challenge studies in SPF broilers as well as field trials where either virus type was the most prevalent. Bursal protection, PCR analysis (viral loads) and performance results will be summarized and discussed.

Deciphering the Mode of Action of MB-1, a Live Hatchery Vaccine against Gumboro Disease

Yossi Wein
Phibro Animal Health

Flow Cytometric Analysis of T Regulatory Cells in the Bursa Of Fabricius Of Chickens Infected With IBDV

Salik Nazki
The Pirbright Institue

Infectious Bursal Disease Virus (IBDV) has a tropism for B cells and causes immunosuppression in chickens. While B-cell destruction is likely responsible for the suppression of humoral immunity, the mechanism underlying the suppression of cellular immunity is poorly understood. Chicken CD4+CD25+ TGFß+ cells have recently been reported to be T regulatory cells (Tregs), and we hypothesized they play a role in IBDV-mediated suppression of cellular effector responses. To address this, three groups of 24 chickens of either 2 days or 2 weeks of age were intranasally inoculated with 100µl of vaccine strain (228E), classic field strain (F52-70) (105 TCID50 / bird), or PBS (mock). Six birds from each group were culled at 7, 14, 28, and 35 days post-infection (dpi). Both 228E and F52-70 led to atrophy of the bursa of Fabricius (BF), a significant reduction of Bu1+-B cells, and a significant increase in CD4+ and CD8+ T cells in the BF. While there was an increase in the expression of TGFß by RTqPCR at all time points studied, CD4+CD25+ TGFß+ Tregs only appeared in the BF at 28dpi. We speculate that alternative sources of TGFß play a role in immunosuppression at earlier time points, and the increase in bursal Tregs at 28dpi might be to maintain immune homeostasis after infection. Interestingly, we also observed an increase in bursal CD4+CD8+ cells following infection. These cells have also been reported to have a suppressor phenotype and, while they did not stain for TGFß, we speculate they may suppress cellular immunity by other mechanisms.

Evaluation of Host Genetic Resistance to Infectious Bursal Disease Virus

Julia Blakey
USDA- ARS USNPRC

Ten lines of MHC-B congenic SPF chickens and two lines of SPF chickens with similar B haplotype but differing non-MHC genes were utilized to investigate the effect of MHC-B haplotype on infectious bursal disease (IBD) incidence. Chickens were challenged at 28 days of age with AL-2, STC, or rA strains of infectious bursal disease virus (IBDV). IBD severity was evaluated throughout a seven-day course of infection based on mortality rates and bursal lesion scoring from H&E bursal sections. Results demonstrated that both the MHC-B haplotype and IBDV virus strain impacted IBD susceptibility. MHC-B congenic lines B*13 and B*19 were consistently in the most IBD susceptible group, while several other B congenic lines were determined as intermediate susceptibility based on survival and bursal scores. IBDV strain rA separated the chicken lines best by mortality, while IBDV strain STC best highlighted differences based on bursal lesions.

ILT/Marek's

Comparison of Marek's Disease Virus Challenge Strains in Commercial Broiler-type Chickens

John Dunn
USDA-ARS-USNPRC

We recently reported on a comparison of Marek's disease virus (MDV) challenge strains in SPF white leghorn chickens for the purpose of meeting immunogenicity testing requirements required by the Code of Federal Regulations for vaccine licensing. For testing a serotype-3 vaccine, a "virulent" label claim requires at least 80% of the unvaccinated chickens to develop lesions, however, multiple companies have reported inconsistency in development of Marek's disease in unvaccinated commercial SPF chickens inoculated with standard challenge strains. In this study we have expanded this evaluation of challenge strains to commercial broiler-type chickens, which are required to be used for licensing vaccines that will be labeled for broiler-type chickens in some countries. We evaluated a total of seven challenge viruses, consisting of both virulent MDV (vMDV) and very virulent MDV (vvMDV) isolates. Several vvMDV candidates were close to the required 80% disease incidence, whereas none of the vMDV strains were near 80%. Results will be discussed from these challenge studies as well as protection studies using a commercial serotype-3 vaccine.

In ovo vaccination with HVT accelerates immunocompetence in chickens

Allison Boone
North Carolina State University, College of Veterinary Medicine and North Carolina Veterinary Diagno

We have previously demonstrated that in ovo vaccination with herpesvirus of turkey (HVT) accelerates the immunocompetence of 1-day-old meat-type chickens and the recommended dose (HVT-RD, 6080 plaque forming units (PFU)) offered the best immunopotentiating effects compared to half-dose (HVT-1/2, 3040 PFU), quarter-dose (HVT-1/4, 1520 PFU), and double-dose (HVT-2x, 12160 PFU). While all the vaccinated groups induced significant splenic lymphoproliferation with Concanavalin A, HVT-1/4, HVT-RD, and HVT-2x increased the percentage of splenic granulocytes at hatch. HVT-RD induced the most pronounced cellular responses, as determined by an increased frequency of splenic T cells (CD3+), including CD4+ T cell subsets, as well as T cells with expression of major histocompatibility complex (MHC)-II. Significant wing-web thickness following phytohemagglutinin-L injection (used to evaluate innate/cell-mediated immune responses) was only noted with HVT-RD, which also significantly upregulated expression of interferon-gamma receptor 2 and Toll-like receptor 3 (TLR3) in the spleen. Results showed vaccine dose had an effect on the innate and cellular responses evaluated and HVT-RD was most ideal. Objectives of the present study were to assess if we could enhance the positive effect of in ovo vaccination with HVT by adding TLR agonists and if this would provide dose-sparing effects. We also sought to evaluate the mechanisms by how in ovo vaccination with HVT influences the immune responses in 1-day-old meat-type chickens and if combining other Marek's disease vaccines with HVT in ovo could affect the immunopotentiating activity of HVT. Results will be discussed.

Efficacy of a new trivalent vHVT-IBD-ILT vaccine administered to day-old pullets against virulent ILTV challenge performed at two time points

Andrea Delvecchio
Boehringer Ingelheim AH

Infectious laryngotracheitis (ILT) is a viral respiratory disease of chickens that affects the poultry industry worldwide. Vaccination is usually applied in high ILTV prevalent areas as an effective strategy to control the disease. The chicken embryo origin (CEO) ILT live attenuated vaccines have been used successfully for many years to control the disease. Nevertheless, although their proven efficacy in protecting against disease and reducing challenge virus replication, they can regain virulence and they have been associated with ILT epizootics worldwide. More recently, safer vectored ILT vaccines, such as the vHVT-ILT, have been developed and are used alone or in combination to CEO vaccines. The objective of this study was to evaluate the efficacy of a new trivalent vHVT-IBD-ILT vaccine administered alone or in combination with CEO against ILTV challenge. Furthermore, the effects of the vHVT-IBD-ILT on CEO replication were evaluated. ILTV challenge virus was administered by intra-tracheal route either at 9 or at 14 weeks of age. The efficacy was evaluated by the ability of the vaccines to protect against clinical signs, to preserve the body weight, and to decrease the challenge virus replication. Results showed that the group that received the CEO in combination with the vHVT-IBD-ILT had a significant reduction of CEO vaccine replication when compared with the group that received CEO alone. After the ILTV challenges, at both ages, the trivalent vHVT-IBD-ILT, alone or in combination with CEO, proved its efficacy in reducing clinal sings, protecting against mortality and decreasing the challenge viral shedding. In conclusion, this study confirmed what was already reported that priming with recombinant HVT-ILT vaccine was able to reduce the CEO replication and secondary reactions. Furthermore, the combination of vHVT-IBD-ILT with a CEO provided a higher level of efficacy when compared to the utilisation of the vaccines alone.

HVT-ILT Recombinant Vaccines: Dynamics of Replication and Protection in Commercial Broilers

Ivan Alvarado
Merck Animal Health

In this study, the levels of HVT replication in feather follicles of two HVT-ILT recombinant vaccines (HVT-ILT A and HVT-ILT B) and their efficacy against early (21 days) and late (40 days) challenge were evaluated.Fourteen-day old embryos were obtained from a 37-week-old commercial broiler breeder flock in Georgia and transferred to an incubator at PDRC, University of Georgia. At 18.5 days of embryonation, eggs were randomly divided in four groups of 30 eggs each. Two groups of 30 embryos were manually vaccinated in ovo with the HVT-ILT A or HVT-ILT B commercial vaccines, following the recommendations of the manufacturers. The remaining two groups were inoculated in ovo with commercial vaccine diluent.Replication of the HVT vector in the vaccinated groups was detected in feather follicles at 14 days of age by and HVT specific qPCR and only positive broilers remained in the study. At 21 and 40 days of age, broilers in the two vaccinated groups and broilers from the positive control group were challenged by intratracheal and eye drop administration of 103.8 TCID50 of the highly virulent 1874C5 strain. The remaining non-vaccinated group was kept as negative control group (non-vaccinated/non-challenged). Efficacy of the HVT-ILT vaccines was evaluated using the following parameters: clinical signs from 3 to 6 days post-challenge, DNA genome load of the challenge strain in tracheas at 3- and 5-days post-challenge and livability for 6 days post-challenge.At 14 days of age, the presence of the HVT genome in feather follicles was detected in 100% and 80% of the broilers vaccinated with the HVT-ILT A and HVT-ILT B vaccines, respectively. The HVT-ILT A vaccine showed significantly higher HVT viral genome load in

tracheas ($p < 0.05$). Increased livability, significantly lower clinical signs scores and significantly lower shedding of the challenge virus was observed in broilers vaccinated with the HVT-ILT A vaccine and challenge at 21 days of age. A significant reduction in clinical signs and 100% livability was also observed in broilers vaccinated with the HVT-ILT A vaccine and challenged at 40 days of age. In contrast, only 60% livability was observed in broilers vaccinated with the HVT-ILT B vaccine.

Comparing ILT Live Vaccine Efficacy Through Drinking Water and Eye Drop by On-Site PCR Test

Keat Fu
Aviagen Inc.

Infectious Laryngotracheitis (ILT) is an important disease in the poultry farm. ILT vaccine can be classified as live attenuated vaccine in embryos or tissue culture, and viral vector recombinant vaccines. The live vaccine can be administrated by eye drops, drinking water or injection. A broiler breeder farm in Taiwan administrate ILT live vaccine by water drinking and eye drops. They use on-site PCR to compare the vaccination uniformity between the two administrations. In this trial, the vaccine strain used is MSD LT-IVAX®, and the breeder age is seven weeks. There are two houses for the trial. They administrate the vaccine via eye drops in house A and administer 1.5 times doses of vaccine via water drinking in house B. Eight samples were collected from trachea swabs on 3, 5,7,11, and 13 days after vaccination from the two houses and analyzed the vaccination uniformity by on-site PCR. In the house A, the ILT positive rates on 3, 7, 11, and 13 days are 50%, 100%, 87.5%, and 37.5%. In house B, and 13 days are 37.5%, 37.5%, 25%, 62.5%, and 62.5%. The positive rates of water drinking are rising slightly slower than eye drops. For this result, it is still acceptable by the customer. They used water drinking instead of eye drops until now and there is no significant clinical sign in the farm. According to that, administrating the ILT vaccine via water drinking in the farm is having acceptable efficacy compared to eye drops.

Lt Serology in Georgia Flocks in the Absence of a Broiler Outbreak

Len Chappell
Georgia Poultry Laboratory Network

A 3,000-sample serology survey of Georgia broilers (>40 days old), broiler breeders (22-26 weeks old and end of life), and commercial layers (35-50 weeks old) was performed to evaluate the LT titers in flocks located in different geographical areas and under different vaccination programs. Statistical analysis was applied to determine any significant differences in these flock populations. All serum samples were tested using the ELISA platform.

Expression of Interferons and Interferon-Stimulated Genes (ISGs) in Larynx, Trachea, and Conjunctiva of Chickens Ocularly Inoculated with Live Attenuated Vaccines and Virulent Strains of Infectious Laryngotracheitis Virus (ILTV)

Daniel Maekawa Maeda
University of Georgia

Interferons (IFNs) play an antiviral role by inducing the expression of interferon-stimulated genes (ISGs). The effect of ILTV vaccination or infection on the expression of host IFNs and ISGs genes is still unclear. The aim of this study was to evaluate the dynamic expression of IFNs type I, II, and III and five ISGs genes in the trachea, larynx, and conjunctiva of chickens after ocular inoculation with the tissue culture origin (TCO) or the chicken embryo origin (CEO) vaccines, or ocular inoculation with ILTV virulent strains 63140 (Genotype V) or 1874c5 (Genotype VI). In the trachea upregulation of type II IFN-? and two to four ISGs tested were upregulated in all ILTV ocular inoculated groups of chickens as

compared to the mock-inoculated controls. In the larynx, type II IFN-? and type III IFN-? and all five ISGs genes tested were upregulated for CEO, 63140, and 1874c5 inoculated groups but not for the TCO group. While type I IFN-ß expression was upregulated in the larynx of CEO and TCO inoculated groups of chickens. Analysis of IFNs and ISGs in the conjunctiva are pending. Expression of IFNs and ISGs genes will be compared to the level of lytic replication and genome load in trachea, and conjunctiva after ocular inoculation with CEO, TCO, 1874c5, and 63140 viruses.

Bacteriology

Campylobacter hepaticus and its Role in the Emergence of Spotty liver disease in Layers

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Spotty liver disease (SLD) occurs in layers around the peak of lay and mainly in free range flocks. It is associated with an egg production drop, increased mortality, and necrotic liver lesions. Specifically brown layers seem to be susceptible for SLD. Campylobacter (C) hepaticus was isolated from livers of layers with SLD, and is considered the causing agent of the disease. The experimental reproduction of the disease has been difficult. Co-factors may contribute to the pathogenesis of SLD. Our objectives were to determine if the chicken genotype may contribute to differences in SLD pathogenesis, and if C. jejuni co-colonization would modify the outcome of the infection. We inoculated groups of four different layer lines (two brown and two white genotypes with the potential of either high or low egg production) at the age of 23 weeks with C. jejuni or placebo. Three days later subgroups received C. hepaticus or diluent. Only one of a total of 101 C. hepaticus-inoculated birds showed a spotty liver. This bird belonged to the brown genotype with low laying performance. Microscopical liver lesions were seen in most C. hepaticus mono- and co-inoculated groups except for the white genotype with low laying performance. C. hepaticus was detected in four to 11% of the animals/group indicating their susceptibility for C. hepaticus colonization independent of their laying performance or C. jejuni co-colonization. Overall, the number of birds developing macroscopical lesions was very low under our experimental conditions pointing out the importance of exacerbating factors in the field.

Does a Post-biotic Product have a Protective Effect Against APEC Challenge?

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Univ of Georgia

Avian pathogenic Escherichia coli (APEC) causes systemic extra-intestinal disease in poultry, known as colibacillosis. Live vaccines and probiotics used to control APEC may be ineffective against the diversity of APEC causing disease. Here, we assessed Diamond Vs XPC postbiotic additive as a gut health enhancer and its potential protective effect against an APEC challenge. In two identical trials, 120 chickens were split into eight groups: four groups were fed a control diet and four groups were fed the XPC diet for 21 days. On day 14, birds were challenged with either Phosphate Buffered Saline (PBS) (control) or APEC O78 (intratracheally or orally) at a concentration of 1X108cfu. On day 21, birds were euthanized and necropsied. Swabs of heart blood, air sacs and tissues of the liver, spleen, lung and ceca were collected for bacterial counts. All birds were scored for evidence of lesions in liver, lungs, air sacs and heart using a standard rubric. APEC was detected in challenged birds fed the control diet at a similar rate to the XPC diet for cecal counts however counts for other organs differed. Lesion scores in challenged birds were consistently

lower in birds on the XPC diet, suggesting that XPC provided some level of protection. The route of challenge resulted in greater systemic disease and significantly higher lesion scores for intratracheally challenged birds compared to oral challenge but the scores were lower in birds on the XPC diet. This study demonstrates potential protective efficacy of postbiotic feed additives on poultry health.

Enterococcus faecalis Modulates Virulence of Avian Pathogenic E. coli by Stimulating Growth and Production of Capsular Polysaccharides

Grayson Walker
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Avian pathogenic E. coli (APEC) and Enterococcus faecalis (EF) are frequently co-isolated from poultry with colibacillosis; however, interactions between these agents as they relate to the pathogenesis of colibacillosis are unknown. Screens of 30 APEC strains grown alone or in mixed culture with EF under iron-limited conditions revealed enhanced growth of the APEC in mixed culture. Comparative genomic analyses revealed APEC capsular polysaccharides and IncF plasmid genes were involved in the interspecies interaction. In vitro experiments were conducted to determine the magnitude of growth and capsule augmentation of an APEC strain and E. coli K-12 laboratory strain in response to EF. Both E. coli strains had enhanced growth when grown in proximity to EF (P 0.05). Curing the APEC strain of its IncF virulence plasmid or deleting a protein kinase regulator of capsule synthesis, rcsC, in the K-12 strain abolished the EF-induced phenotype. In vivo embryo lethality experiments demonstrated that, while co-infections of wild-type E. coli K-12 and EF were lethal to chicken embryos, co-infections of a ?rcsC isogenic mutant K-12 strain and EF were avirulent (P < 0.05). RNA was isolated from APEC and E. coli K-12 grown with and without the influence of EF signals for differential gene expression analysis, which will be presented. Taken together, these findings show that a widely conserved virulence factor of APEC, capsule production, is augmented by EF which may play a role in systemic infections in poultry. Further work, including treatment and management approaches that disrupt these pathogen interactions, are warranted to better manage colibacillosis in poultry production.

Identification of Novel Genes Involved in the Biofilm Formation Process of Avian Pathogenic Escherichia coli (APEC)

Meaghan Young
University of Georgia

Avian pathogenic Escherichia coli (APEC) is the etiological agent of avian colibacillosis, a leading cause of morbidity and mortality in the poultry industry worldwide. Biofilm is an important factor for APEC survival. Since much is still unknown about the genetic factors of biofilm formation, the objective of this study was to identify novel genes involved in the biofilm formation ability of APEC. A total of 15,660 mutants of a well-characterized APEC serogroup O18, ST95 strain (APEC 380) were randomly created using the signature tagged mutagenesis technique and evaluated for decreased biofilm formation ability. Mutants with a >50% decrease in biofilm formation ability compared to the wild type were sequenced around the transposon insertion and analyzed with BLAST-N for putative biofilm formation genes. A total of 547 putative biofilm formation genes were identified, including genes already known to be involved in biofilm formation and those not known. To determine which genes were most important in APEC, 30 of the identified genes were analyzed via PCR for prevalence in 109 APEC and 104 avian fecal E. coli (AFEC) isolates. A total of 9 genes had significantly higher prevalence in APEC than AFEC isolates. The presence of these genes in APEC at a significantly greater rate than AFEC suggests that these genes are important in forming APEC biofilms and can be used as

potential targets for antimicrobials and other therapeutics without disrupting commensal E. coli. Further research will evaluate the importance of these genes throughout different phases of biofilm production.

Early turkey mortality related to infection with Streptococcus gallolyticus

Ann Wooming
Church and Dwight

Early turkey mortality related to infection with Streptococcus gallolyticusAnn Woominga, Xandra Smitha, Jodi Delagoa, Zachary ZawadaaArm & Hammer Animal and Food ProductionaSeveral commercial turkey companies reported increased mortality in birds between 2- 3 weeks of age. Clinical signs included splenomegaly, hepatitis and occasional pericarditis. Affected flocks include hens and toms and were spread across the Midwest to the East coast. Clinical isolates (n=54) were recovered from livers, spleens, or small intestines from affected turkeys from seven different producers across the affected region. Sequencing the 16S rRNA gene of the isolates identified Streptococcus gallolyticus as the causative agent. Genetic diversity of S. gallolyticus isolates recovered from diseased turkeys was assessed using random amplified polymorphic DNA-PCR (RAPD-PCR) analysis. Comparison of the RAPD-PCR profiles from each isolate indicated two main clades among the isolates analyzed. Isolates from individual producers tended to fall into both clades indicating that both main RAPD types have a broad geographic distribution. However, isolates recovered from intestinal tracts from one producer were limited to a single clade, suggesting a clonal population within the single flock that these isolates were recovered from. The increased incidence of mortality due to infection with S. gallolyticus poses a serious challenge to turkey producers, especially those involved in antibiotic free (ABF) and no antibiotics ever (NAE) programs.

Genotypic Classification of Avibacterium paragallinarum, the Causative Agent of Infectious Coryza

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Infectious coryza is an upper respiratory disease of chickens caused by the bacterium Avibacterium paragallinarum. Currently, there are two classification methods for A. paragallinarum using hemagglutination inhibition tests. The Page scheme divides strains into serogroups A, B and C, while the Kume scheme subdivides serogroups A, B and C into 9 serovars: A-1 to A-4, B-1, and C-1 to C-4. Both assays are complex, expensive, and performed in very few laboratories. To overcome this challenge, we developed a molecular classification method that corresponds to the Kume scheme targeting the HMTp210 gene. This gene corresponds to a membrane protein with hemagglutinating capability. The phylogenetic analysis is composed of one portion of the gene that classifies all strains into A, B, C-1, C-2, C-3, and C-4, and a second portion that classifies the serogroup A strains into serovars A-1, A-2, A-3, and A-4. This novel classification method is an alternative to the serotyping taxonomy and provides information that might aid surveillance, prevention, and vaccine development for infectious coryza.

Development of Core Genome Multilocus Sequence Typing (cgMLST) Scheme for Genotyping of Pasteurella multocida

Mohamed El-Gazzar
Iowa State University

Characterization of Pasteurella multocida (PM) is essential to the prevention, control and eradication of Fowl Cholera (FC) from poultry flocks. The conventional method for PM

characterization is serotyping based on somatic antigen, which is labor intensive and often results in ambiguous serotypes. Additionally, PM serotyping is only available in a few laboratories which results long waiting times. Molecular methods are used in typing PM including Pulsed-Field Gel Electrophoresis (PFGE) and conventional Multilocus Sequence Typing (cMLST). PFGE is the most used genotyping method; however, it also is not widely available in diagnostic laboratories. This often leaves the poultry industry unable to characterize most PM outbreaks. Recently, whole genome sequencing data is becoming available and readily accessible to more diagnostic laboratories. The objective of this study is to capitalize on the availability of whole genome sequences to develop and validate core genome Multilocus Sequence Typing (cgMLST) for PM. By studying the whole genome sequences of a diverse set of PM isolates, conserved core genes present in all member of the species were identified. These core genes were then used to characterize and type PM isolates into epidemiologically related types. Typing results from this assay can be easily shared and compared between laboratories across the world which in turn would lead to a much improved understanding of the epidemiology of FC and better prevention, control and eradication methods.

Case Report: Is The Prevalence of Clostridium Septicum Dermatitis and Septicemia Underestimated in Commercial Egg Layers?

Michaela Olson
Wilson Veterinary Company

Over the last two years, our practice has encountered a number of unusual septicemia and dermatitis in flocks with elevated mortality attributed to Clostridium septicum infection. The first case that was presented at one of our farm locations was an unexpected discovery, as it was initially written off as post mortem decomposition in a caged layer flock. The actual cause of death was discovered only after a diagnostician at a state diagnostic lab with experience in broiler gangrenous dermatitis suggested anaerobic culture. Flocks present with a variety of lesions that can easily be confused with post mortem decomposition and bad mortality management in a caged layer house. On closer inspection some birds have mild to moderate serosanguinous subcutaneous fluid with or without crepitus gas pockets. Some individual birds also have internal lesions consistent with ante-mortem gas pockets in the liver and spleen. Ultimately, aseptically sampled bone marrow of these birds shows heavy growth of Clostridium septicum. Affected flocks respond to treatment with chlortetracycline by veterinary feed directive, which does lead to a decrease in mortality. The presentation of these cases makes us wonder - is the prevalence of multi-organ clostridial septicemia underestimated in caged layers? It has not been a traditional practice to perform anaerobic cultures on routine layer mortality submissions in the absence of clearly discernible dermatitis lesions. This case report will cover further details from affected flocks and discuss the possibility that more cases may be overlooked by field veterinarians and diagnosticians that easily write mortality causes off as post mortem decomposition.

Assessing Two Fertile Eggshell Interventions With Luminometry

Ricardo Munoz
Neogen Corporation

Hatchery biosecurity's potential highest risk of contamination could be fertile eggs. In many operations around the world, one of the most critical points is deciding whether or not to include fertile eggs that, after visual inspection, do not demonstrate a clear absence of organic matter or color indications that may lead to potential contaminations, as well as the collection of apparent clean eggs that may have

been found out of the nest.In this field study, two groups were evaluated, with each group having 4,690 eggs. One group was designated as Nest Group, which has been exposed in a close room with a paraformaldehyde-based gas for 20 minutes, and the second as the Floor Group, which was treated with a peracetic acid solution at 37 Celsius. The two groups were compared in terms of eggshell ATP (Adenosine Triphosphate) presence, and the regular data was collected for mesophiles and coliforms in the yolk sac and embryo-internal organs at the embryo-diagnosis.No statistical differences were found in terms of eggs contaminated from each group: N=42/F=39 from the total of each group.The fertile egg's surface represents a continuous challenge for hatchery biosecurity and the hygiene status of hatchery surfaces. Various factors may affect the hygiene quality of the eggshells of fertile eggs, including nest material, nest management, percentage of eggs laid on target, feces contamination, etc.

Stress during Rearing-Laying Transition changes Gut Microbiota Profile, Intestinal Cytokine Expression and Skeletal Properties in Commercial Laying Hens

Prafulla Regmi
University of Georgia

Commercial cage-free laying hens are often reared in large numbers in housing environments that have complex designs. In order to thrive in a physically and socially complex production environment for longer than 75 weeks, a hen should be able to maintain physiological homeostasis. Despite best efforts, a number of welfare issues are observed in cage-free systems High incidences of keel bone fractures, and mortality associated with opportunistic bacterial and protozoal infections are the major health and welfare problems in cage-free houses. These welfare issues are often considered to be predisposed by stressful insults (catching, handling, thermal, transportation). A comprehensive understanding of the common pathways or the framework through which various stress and risk factors act in the development of welfare issues is lacking. In this study, effects of short-term exposure to catching, handling and transportation stress at 17 wk (end of pullet phase) was studied in brown egg-type layers. Pullets were reared in floor houses with 320 birds per room with 4 rooms used for the experiment. Birds from 2 rooms (AC) were transferred to 4 sections (144 hens/section) of a multi-tier aviary house at 17 wk whereas the birds from the remaining two rooms were continued in the floor system during lay (FC). Eight birds per treatment (2 per section from AC group; 4 per room from FC group) were randomly sampled at 18, 20, and 24 weeks for cecal microbiota profile, immune cytokine gene expression and bone quality parameters. Differentially abundant taxa as indicated by linear discriminant analysis was significantly different between FC and AC birds (P 0.05). The results indicate that transition from pullet phase to hen phase induces significant stress to cause gut dysbiosis and alter bone properties.

HPAI Focus

Epidemiology of the 2022 H5N1 HPAI outbreak in the U.S.

Julie Gauthier
USDA

Beginning in February 2022, outbreaks of H5N1 HPAI caused severe disease and very high mortality in more than 200 flocks spread across most U.S. States. Emergency response activities involved depopulation and disposal of tens of millions of birds, cleaning and disinfection of affected farms, surveillance, and movement restrictions for poultry and poultry products. Epidemiologists are currently analyzing the spread of virus, identifying risk factors for flocks

becoming infected, and revealing emerging trends in transmission.

Vaccine Options for Highly Pathogenic Avian Influenza in the United States

David Suarez
USDA

Update on HPAI in the US

Mia Torchetti
National Veterinary Services Laboratories

In January 2022 H5N1 clade 2.3.4.4b highly pathogenic avian influenza (HPAI) was detected wild migratory waterfowl in the Atlantic flyway following reports of the virus in poultry in Newfoundland. By June 2022, HPAI had been confirmed in 226 poultry premises and 146 non-poultry flocks across 36 states, and over 1500 wild bird detections among 68 species across 42 states including Washington D.C. and Alaska. In collaboration with Wildlife Services and ARS Southeast Poultry Research Laboratory, analysis of full genome sequences from wild birds and poultry was conducted to help determine virus origins, reassortments, and epidemiology. Reassortment between H5 2.3.4.4b and North American lineage influenza A virus became predominant by April-May. By late April, the first mammals (young red foxes) were reported and were subsequently detected across several northern and midwestern states from coast to coast. An overview and summary of findings will be presented.

AAAP Posters

Antimicrobial/Antibiotic Resistance

Minimal inhibitory concentrations of avilamycin to Clostridium perfringens isolates from broiler chicken farms before and after the approval of Surmax® Premix (Avilamycin) in Canada

Eric Parent
Elanco Canada

Avilamycin, the active ingredient in Surmax® Premix, is a non-medically important (animal-only) in-feed antibiotic used for the prevention and control of necrotic enteritis (NE) associated with Clostridium perfringens in broiler chickens, an economically significant disease in the poultry industry. The ongoing efficacy of Surmax® Premix is therefore critical to reduce the reliance on shared class antimicrobials between poultry and human medicine as a key part of the strategy to limit the development of antibiotic resistance to human use antibiotics. Minimal inhibitory concentrations (MICs) of avilamycin were determined in 89 isolates of C. perfringens recovered from NE field cases pre-avilamycin approval (n=50, 2003 to 2013) and post-avilamycin approval (n=39, 2014 to 2021) in Canada. MICs were determined by the agar dilution method following the Clinical and Laboratory Standards Institute (CLSI) recommendations for anaerobic bacteria (test range 0.0625-32 µg/ml; 10 dilutions). MICs ranged from 1 to 8 µg/ml before avilamycin approval and from 0.5 to 2 µg/ml after avilamycin approval. Pre-avilamycin approval MIC50 and MIC90 were both 2 µg/ml. Post-avilamycin approval, MIC50 corresponded to 1 µg/ml and MIC90 was 2 µg/ml. These results indicate that antibiotic susceptibility to avilamycin was not affected by its use following the registration of Surmax® Premix in Canada for the prevention and control of NE associated with C. perfringens in broiler chickens. This study also provides novel data showing low MICs for avilamycin in C. perfringens isolates recovered from commercial flocks, indicating that field cases of NE may have been prevented and controlled using this animal-only antibiotic.

The protective role of Coli-vac, a heptavalent vaccine against extraintestinal avian pathogenic Escherichia coli (APEC) infection

Wael Elfeil
Suez Canal University

Infection of poultry flocks with avian pathogenic Escherichia coli (APEC) leads to colibacillosis, a serious disease that affects all ages and heavily impacts the economy of poultry production worldwide. During November and December 2020, 36 APEC isolates were identified and serotyped in Egypt. They belonged to 3 major serogroups; 4, 2 and 3 and involved serotypes O27, O78, O125, O126, O166 and O157:H7. More than 80% of these isolates showed multiple drug resistance (MDR) to 8 different antibiotics and all showed 60-100% mortality in day-old chickens. Seven strains were used to develop a multivalent inactivated vaccine containing an oil adjuvant. Groups of 1- and 7-days old SPF chickens were used to assess the vaccine safety, immunogenicity, and efficacy. Challenge was conducted 2 weeks after vaccination of each group utilizing homologous (O157:H7-serotype 3) and heterologous APEC strains (O125-serotype 2). The vaccine proved safe and produced high agglutination antibody titers. Protection reached 90% and 85% against homologous and heterologous challenges, respectively. Non-vaccinated control chickens developed severe clinical signs in 60-70% of the birds after challenge. The results highly suggest the use of this vaccine in the control of colibacillosis, which along with good farming and

biosecurity practices could decrease the unrestrained use of antibiotics in the treatment of this disease and subsequent public health threats due to the development of antimicrobial resistance.

Chicken Gut Microbiota Dynamics after Amoxicillin and Thiamphenicol Treatment

Andrea Laconi
University of Padua

We aimed at investigating the selective pressure exerted by Oraladministration of amoxicillin and thiamphenicol on chicken's gut microbiota. Eighteen broiler chicks were allocated in three groups (six birds per group): one without any antibiotic treatment (control group) and two treatment groups, one treated with amoxicillin and one with thiamphenicol, both at 5 days of age for three consecutive days. Cloacal swabs were taken from all birds at 1 day of age and then on days 8, 19, and 28. The microbiome was determined by NGS employing a 16S rRNA gene amplicon sequencing approach using Miseq platform (Illumina). Raw sequence data were analysed with QIIME 2, while Silva database and Calypso software were employed for taxonomical and statistical analyses, respectively. a-diversity was comparable between groups at the same time-point. Meanwhile, ß-diversity analysis showed spatial separation between treated and untreated groups. Several genera were significantly reduced after treatments with amoxicillin and/or thiamphenicol (e.g. Helicobacter, Streptococcus and Bifidobacterium), other increased (e.g. Gallibacterium, Bacteroides, and Sphingomonas). Core genome analysis identified six persistent taxa shared by at least 80% of the individuals per group per time-point (e.g. Enterococcus, Escherichia/Shigella, and Lactobacillus). Our findings seem to suggest that treatments with amoxicillin and thiamphenicol may influence the abundance of specific taxa in the chicken's gut microbiota.

Avian Influenza

Infection of H5N6 HPAIV and evaluation of inactivated H5N6 HPAI vaccine on ducks

Seojeong An
Konkuk University of Veterinary Medicine

In the winter season of 2016-2017 the outbreak of clade 2.3.4.4 H5N6 highly pathogenic avian influenza virus caused devastating damage in South Korea poultry industry. The A/duck/Korea/ES2/2016 H5N6 virus (ES2) was isolated from ducks during this outbreak. The infection of ES2 virus caused mortality and viral shedding in 5-weeks old ducks. The bird infectious dose 50 (BID50) of the virus was less than 104EID50 and the bird lethal dose 50 (BLD50) was 104.74EID50. 1 and 1/10 dose of oil-emulsified inactivated ES2 HPAIV vaccine provided enough serum antibody hemagglutination inhibition titer. 1, 1/10 and 1/50 dose of the vaccine protected ducks from lethal challenge of the virus, but only 1 dose of the vaccine significantly suppressed oropharyngeal and cloacal viral shedding in ducks. These results indicate that oil-emulsified inactivated ES2 HPAI vaccine can reduce viral shedding and protect domestic birds from ES2 HPAIV infection.

Antigenic Characterization of Low Pathogenic Avian Influenza Virus H9N2 isolated in the Republic of Korea

Andrew Cho
Konkuk University

Low pathogenic avian influenza virus (LPAIV) H9N2 has consistently caused outbreaks in poultry farms in Asia, Europe, and Africa. H9N2 LPAIV in poultries have evolved in mostly three different lineages: Y280-like lineage, Y439-like

lineage, and G1-like lineage. Historically, Y439-like lineage viruses, also known as, Korean-lineage viruses have been endemic in South Korea. In 2020, Y280-like lineage H9N2 LPAIV was detected in a live poultry market in Korea for the first time. Since then, Y280-like lineage H9N2 LPAIV has been affecting mostly layers and LBM poultries with little to no biosecurity since its introduction in South Korea. In this study, we examined the antigenic variation among 20 H9N2 LPAIV.H9N2 LPAIVs isolated in South Korea from 1996 to 2021 and the vaccine strain currently used in South Korea to control H9N2 LPAIV was used to produce reference antiserum in chickens. Antiserum was used to determine the antigenic distance among the viruses. Antigenic cartography was generated using the hemagglutination inhibition (HI) assay of the 20 viruses. Antigenic cartography showed that there is evidence for varying degree of antigenic distances between these strains. The three-dimensional structures of H9N2 LPAIV hemagglutinin protein highlighted the antigenic sites that may have caused the antigenic distance between the strains. Also, molecular analysis of the newly endemic H9N2 LPAIV revealed the presence of mammalian host-specific markers.The newly endemic Y280-lineage H9N2 LPAIV pose public health threats to poultry industry workers. Antigenic variation among the H9N2 LPAIV calls for continuous monitoring, adaptive vaccination strategy, and appropriate biosecurity measures.

Evaluation protection of H5 vaccination regimes against early challenge with HPAI-H5N8 Clade 2.3.4.4

Wael Elfeil
Suez Canal University

The aim of this work was to evaluate effectiveness of inactivated H5 vaccine in different vaccination regime either as single vaccine dose at 7-day of age, two-dose regime (1/10-day of age) or using as one and half manufacture recommended dose at 7-day of age against early challenge at 28-day of age with HPAI-H5N8 clade 2.3.4.4 virus. 75,000 one-day old chicks obtained from commercial hatchery placed in three commercial broiler station “25,000 birds/station” (G-1 two-dose regime, G-2 one and half dose regime and G-3 single-dose regime) and 40 one-day old chicks moved to Biosafety level-3 isolators (BSL-3) at MEVAC facility to serve as control groups (non-vaccinated group challenged “G-4” and Non-vaccinated non challenge group “G-5”). On weekly basis blood samples, cloacal swabs and oropharyngeal swabs collected check develop of HumOralimmune response and exposure to any life-threatening Respiratory virus (Avian influenza” AIV”, Newcastle disease “NDV” and infectious Bronchitis “IB” virus). At 26-day of age 25 birds from each station (G1-3) moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs collected and three birds from each group euthanized, and internal organs examined for three repeated times with 12 hours interval to ensure that birds free from any live threating viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 challenged with HPAI-H5N8 clade 2.3.4.4 (106 EID50) in 0.5 ml/ bird PSB via intranasal route and birds in G-5 received 0.5 ml PBS via intranasal route. Birds in G1-5 kept in BSL-3 for 10 days under observation and oropharyngeal swabs collected on 3,6,9 days post challenge (dpc). Regarding protection virus against mortalities following challenge with HPAI-H5; were 100% (12/12), 91.7% (11/12), 91.1% (11/12), 0% (0/12) and 100% (12/12) in groups 1-5 respectively. Regarding virus shedding birds in G-1 showed significant lower shedding virus (amount of virus shedding and number of shedding in comparison to G2/3. In conclusion, using homologous H5 inactivated vaccine in two-dose regime can provide protection to commercial broiler chicken against early challenge with HPAI-H5N8 clade 2.3.4.4 virus as early as 28 days of age, with significant

lower shedding rate: the application of such regime can be an effective tool to control HPAI under a vaccination strategy.

Live Recombinant NDV-Vectored H5 Vaccine Protects Chickens and Domestic Ducks from Lethal Infection of the Highly Pathogenic H5N6 Avian Influenza Virus

Heesu Lee
Konkuk University, Seoul

The H5 subtype highly pathogenic avian influenza virus (HPAIV) have been introduced to South Korea every two or three years via wild migratory waterfowls, causing devastating damages to the poultry industry. Although most damages and economic losses by HPAIV are focused on chicken layers, domestic ducks are known to play a major role in the farm-to-farm transmission. However, most HPAIV vaccine studies on poultry have been performed with oil-emulsion inactivated vaccines. In this study, we developed a live recombinant Newcastle disease virus (NDV)-vectored vaccine against H5 HPAIV (rK148/ES2-HA) using a previously established NDV vaccine strain (K148/08) isolated from a wild mallard duck. The efficacy of the vaccine when administered via the oculonasal route or as a spray was evaluated against lethal H5 HPAIV infection in domestic ducks and chickens. Oculonasal inoculation of the rK148/ES2-HA in chickens and ducks elicited antibody titers against HPAIV as early as 1 week after the single dose of vaccination, whereas spray vaccination in ducks elicited antibodies against HPAIV after the booster vaccination. Furthermore, vaccination in ducks tolerated pre-existing antibodies against NDV, which were presumably derived from breeder ducks. The chickens and ducks vaccinated with rK148/ES2-HA showed high survival rates and low viral shedding after H5N6 HPAIV challenge. Collectively, vaccination with rK148/ES2-HA prevented lethal infection and decreased viral shedding in both chickens and ducks. The vaccine developed in this study could be useful in suppressing virus transmission in H5 HPAIV outbreaks, with the ease of vaccine application and fast onset of immunity.

Stepwise adaptation of Hemagglutinin of H5N2 Highly pathogenic avian influenza virus in chickens

Sungsu Youk
USDA ARS US Natl Poultry Research Ctr

Stepwise adaptation of Hemagglutinin of clade 2.3.4.4 H5N2 Highly pathogenic avian influenza virus in chickens

Bacteriology

Molecular characterization of few Avibacterium paragallinarum isolates from different US states using whole genome sequencing

Mostafa Ghanem
The Ohio State University

Avibacterium paragallinarum (AP) is a gram-negative, non-motile bacterium that causes Infectious Coryza (IC), a respiratory disease of chicken. Recently, there has been an increased prevalence of IC in commercial and noncommercial chickens including layers and broilers in the Northeast United States. Next-Generation sequencing technologies facilitated the generation of whole genome sequences of different pathogens at unprecedented rate and lower cost. It allowed higher in-depth studies of bacterial genomes with characterization of antimicrobial resistance profile, virulence factors as well as more accurate phylogenetic analysis of different isolates. Following the recent increased incidence of infectious Coryza in different states, a few number of AP isolates have been successfully isolated in state diagnostic laboratories. In order to understand the genomic characteristics of these isolates, we have generated whole genome sequences of these isolates using Illumina Miseq. The generated

sequences were quality checked, assembled, annotated and analyzed to determine the antimicrobial resistance, virulence factors. In addition, comparative genomic analysis was performed to identify differences and similarities within these genomes and their degree of relatedness. The results of this analysis will be presented in the meeting. This work should improve our understanding of the main genomic features of AP isolates circulating in few US states, Moreover, information about the antimicrobial resistance profile of these isolates could guide antimicrobial choice by practicing veterinarians.

Epidemiological Surveillance Of Chlamydophila Psittaci In Wild Birds From Perú

Rosa Gonzalez
San Marcos University, Lima-peru

Wild birds are recognized as a reservoir of many of bacterial, parasitic and viral zoonotic agents, affecting the poultry industry and public health. The present study aimed to detect by serological and molecular methods, the presence of Chlamydophila psittaci in Psittaciforms birds from Peru. Between June 2020 and August 2021, a total of 26 serum samples and 36 cloacal swab samples from rescue birds of the following species were collected: Ara macao, Ara ararauna, Ara chloroptera, Ara severa, Psittacara mitratus. Two other species were analyzed only by PCR: Orthopsittaca manilatus and Amazona farinosa. In addition, a sample of toucan of the Ramphastos tucanus also was serologically evaluated. The detection of antibodies against Chlamydia psittaci, was conducted using the modified ELISA test with the commercial ImmunoComb Avian Chlamydophila psittaci kit (Biogal). Fifteen samples (Ara macao, Ara chloroptera and Psittacara mitratus) were positive for antibodies against Chlamydophila psittaci, 02 samples (Ara macao) were suspicious and 09 negative samples. The PCR test was carried out following the protocol described by the OIE, 2018. The genetic material of Chlamydophila psittaci was not detected in none of the 36 samples analyzed.The presence of antibodies against Chlamydophila psittaci suggests that the birds have been exposed to the bacteria, indicating the need to continue with sustained epidemiological surveillance for the isolation and detection of the agent.

Does the Nutritionist have a role to play in Managing Campylobacter jejuni in Broilers?

Matthew Jones
Southern Poultry Research Group

Multiple field intervention strategies targeting a lower number of Campylobacter coming into the processing plant may be critical for success in meeting FSIS performance standards. Even nutrients provided to the birds may be useful to reduce these counts. The nutrient density of the diet has direct influence on broiler performance regardless of the environmental conditions. Not only does the diet impact performance, it can also alter intestinal microbial populations. Campylobacter jejuni (CJ) is a leading cause of foodborne illness in people. Campylobacter grows vigorously in poultry and there are currently few products available to control these populations. In vitro, Campylobacter grows in specific media which accommodates the bacteria's predilection for specific nutrients. Using the same logic, can the nutrient profile be altered in vivo to control the proliferation of Campylobacter? In a battery cage experiment, Campylobacter colonization in the ceca will be compared between two dietary treatment groups: a high crude protein diet and a lower crude protein diet. Additional crystalline amino acids will be included in the lower crude protein diet to formulate these two dietary treatments. As crystalline amino acids are added, it takes pressure off the soybean meal (SBM) inclusion, so the level of SBM drops correspondingly. Effectively, the "excess" nutrients provided in SBM are no longer available to the bird. The

dietary treatments will be offered ad libitum for the duration of the experiment. Male Ross broiler chicks will be separated into cages. These cages will serve as the experimental unit. On day of test 14, all chicks will be gavaged with Campylobacter jejuni (~1 x 106CFU/bird). Ceca will be collected from one chick in each cage on day 21 and 28 of the experiment. Mean direct Campylobacter counts from the ceca will be compared between treatments.

High Mortality Outbreaks in Broiler Chickens Caused by Highly Virulent and Multidrug Resistant Escherichia coli

Christopher Poulos
Animal and Plant Health Agency

Between 2020 and 2021 in the Great Britain, there were four outbreaks of high mortality on broiler farms due to peracute to acute colisepticaemia. These farms were located in three distinct geographic areas. In birds greater than 12-days-old, the clinical presentation included a rapidly escalating mortality that was unresponsive to most antibiotics. The most consistent post-mortem findings were hepatomegaly and splenomegaly. Notifiable disease was considered in three of these outbreaks and later negated. As part of the scheme in England and Wales to investigate cases that may mimic notifiable disease, an extensive investigation was undertaken by Animal and Plant Health Agency. Bacteriology detected colisepticaemia in all of examined carcases. Histopathology revealed wide spread dissemination of bacteria and thrombosis. Next generation sequencing was completed on viscera collected from the first outbreak and no significant underlying infectious process was detected. Whole genome sequencing was undertaken on E. coli isolates from each outbreak. With the exception of one isolate, all of the E. coli were identified as the same multilocus sequence type (ST-1564) and shared the same H21 antigen. They contained an overlapping array of virulence genes that are commonly detected in the avian pathogenic E. coli. There were a number of additional genes for virulence that were associated with other pathotypes. Isolates from each outbreak also carried resistance genes for multiple classes of antibiotics. This combination of virulence factors and resistance genes would account for severe clinical presentation and limitations of antibiotic treatment. The overall investigation highlights the emerging significance of a virulent E. coli strain and its implications for animal welfare and one health.

Case Reports

Isolation and Characterization of Goose Tembusu Virus in Taiwan

Hui-Wen Chen
National Taiwan University

Since 2010, Tembusu virus (TMUV) has caused severe outbreaks with neurological signs and egg-drop diseases in ducks and geese in neighboring countries of Taiwan. In late 2020, an infectious disease outbreak characterized with white diarrhea, depression, lameness, prostrate and increased mortality occurred in a 45-day-old white Roman geese flock in southern Taiwan. TMUV infection was diagnosed from diseased geese by RT-PCR. TMUV was successfully isolated using minimal-pathogen-free duck embryos and designated as NTU/C225/20. The full-length genomic sequence of virus was determined by the next-generation sequencing. Genomic analysis revealed that the NTU/C225/20 shares approximately 87% nucleotide identity with recently reported TMUV strains in China, Malaysia and Thailand, and 91% with the prototype strain MM1775 identified back in 1955. In addition, TMUV NTU/C225/20 can be cultured and titrated in cells and embryos, where the virus was able to generate cytopathic effect (CPE) in the chicken DF-1 cell

line and primary duck embryo fibroblasts, and cause death in specific-pathogen-free chicken embryos. Mammalian Vero cells also supported the viral growth in the absence of CPE, and the viral envelope protein can be visualized using anti-flavivirus antibodies in the immunofluorescence assay. Anti-TMUV serum neutralization assay has been established for serological surveillance purpose, and domestic waterfowls including duck and geese serum samples have been collected and examined. In this study, for the first time, a novel TMUV strain from geese of Taiwan was isolated and characterized. Further studies on the infection prevalence and viral pathogenicity are warranted.

Imposter false layer syndrome

Emily Pittman
Georgia Poultry Laboratory Network

Objective—Describe an unusual presentation of dehydration in broiler breeder pullets.Design—Case report.Animals—Female broiler breeders, 23-weeks-old, one house on a four-house farm.Procedure—Six birds were submitted for necropsy for elevated mortality and lethargy. Husbandry was reviewed. Necropsy, histopathologic examination, serological investigation, microbial isolation, and virus detection were performed.Results—Broiler breeders were submitted with a one-day history of elevated mortality and a several days history of lethargy, weakness, and lateral recumbency. Necropsy revealed enlarged, hemorrhagic proventriculus with gel-like substance on the luminal surface. Several had grossly cystic left oviducts. The kidneys were enlarged and pale in three birds. Multiple pullets had yellow nodules throughout the abdominal air sacs, identified as Aspergillus fumigatus. No bacterial or viral agents were identified. Histopathological examination showed nephritis as well as extensive necrosis and hemorrhage affecting the proventricular glands, which is consistent with dehydration. There was loss of glandular structure in the oviducts, consistent with false layer syndrome seen in disease caused by infections with several types of infectious bronchitis virus.Conclusions—This was initially believed to be false layer syndrome caused by a variant of IBV, however, no IBV was detected in any tissues submitted. The histopathology lesions in the proventriculus were consistent with water consumption after dehydration.

Coccidiosis

Sensitivity of Eimeria Field Isolates in the United States: An update on responses to current practices

Luis Gomez
Phibro

Several studies were conducted between 2017 and 2021 to assess the anticoccidial sensitivity (AST) of contemporary coccidia field isolates to several chemically synthesized anticoccidials (and some ionophores or combinations). Isolates were collected from several broiler-producing regions in the United States, propagated once in the absence of anticoccidial medication, and then used to inoculate broilers that were fed nonmedicated rations and those containing the anticoccidials. Although these studies evaluated the performance responses to these anticoccidials, we relied on the average lesion score per intestinal location and the proportional reduction to controls to assess sensitivity. Results of these AST indicated that some drugs retained their relative effectiveness even after prolonged use but there is a clear differentiation between location that rely solely in chemotherapy against those that use a biological control.

Alternatives Treatments for Coccidiosis and Necrotic Enteritis Management

Greg Mathis
Southern Poultry Research, Inc.

Alternatives Treatments for Coccidiosis and Necrotic Enteritis Management Greg F. Mathisa aSouthern Poultry Research, Inc., 96 Roquemore Road, Athens, GA 30607 USAWith the demand for poultry 'Raised Without Antibiotics', the number of alternative products used for controlling coccidiosis and/or necrotic enteritis has greatly increased. This has led to the development and use of several products such as probiotics, prebiotics, essential oils, organic acids, saponins, and tannins. Just as each alternative category varies greatly, within each category there can be large differences. Variation in species composition, strains, activity levels, encapsulation processes, and purity can all contribute to efficacy. These alternatives also vary is their ability to be stand alone or supportive products. Supportive can be with vaccines or anticoccidial drugs. Poultry coccidia vaccinated often benefit from using alternatives; however, some alternatives may inhibit immunity development. If the alternatives do inhibit immunity, it is necessary to determine when to start administration and duration to maximize performance. Understanding each of these differences is critical to selecting the most effective alternative product.

Intestinal Integrity (I2) Correlations to Chicken Gut Health

Francene Van Sambeek
Elanco Animal Health

Elanco Animal Health developed a Health Tracking System (HTSi) for tracking lesions at broiler posting sessions. This data was analyzed using an equation to calculate the Intestinal Integrity (I2) score as a summary of bird health. This talk will look at using I2 as a predictor of gut health outcomes.

Diagnostics

Utilization of the i-STAT1 handheld clinical analyzer and VetScan2 to compare blood values in normal vs. recumbent turkey hens at point of lay

Jewell Bremer
Select Genetics

A plethora of research has been done comparing two widely used blood analyzers to determine blood gas, biochemistry and electrolyte parameters for multiple avian species (Sauer et al., Martin et al.). While much of this research has been performed in commercial broilers and egg laying hens, Schmidt et al., has paved the way with serum biochemical parameters during lay in female bronze turkeys. However, little is known of the blood biochemical parameters on modern broad breasted turkey breeding stock. The objective of this study was to create reference values in modern broad breasted turkey breeder hens utilizing the i-STAT1 handheld fitted with the CG8+ cartridge and VetScan2 analyzer using the avian and reptile rotor. Then compare the blood parameters of normal turkey hens to recumbent hens prior to and at the point of lay to demonstrate associations with paralysis in laying turkey hens.

Evaluation of Flongle and MinION Flow Cells for the Whole-Genome Sequencing of Avian Influenza A Virus in Clinical Samples

Iryna Goraichuk
USDA/ARS/SEPRL

To date, the emergence of novel H5 and H7 highly pathogenic avian influenza (HPAI) viruses have been occurring through two main mechanisms: the conversion of a low pathogenic into a highly pathogenic virus and the reassortment between different genetic segments of currently circulating low and highly pathogenic viruses. The Flongle is a more accessible, cost-efficient single-use flow cell adapter for Nanopore's portable MinION and desktop GridION devices. We compared the

performance of Flongle and MinION flow cells for the whole-genome sequencing of clinical samples in conjunction with in-house barcoded primers for the amplification of H5 and H7 AI genomes.With this aim, SPF birds were experimentally infected with a high dose (106 EID50/0.1ml) of seven different HPAI H5 and H7 strains. Oralswabs were collected at 2 days post-infection. Viral titers in each sample were determined by qRT-PCR and ranged from 3.2 to 7.5 log10EID50/ml. Barcoded amplicons of 7 HPAI were pooled together in equal volume and used for the preparation of two Nanopore libraries to be sequenced using Flongle and MinION R9 flow cells. In just 1 hour of Flongle and MinION sequencing, the total reads quantity was 53,908 and 333,987, respectively. One sample with the lowest viral load was undetectable using both Nanopore flow cells. In the rest of the samples, viruses were successfully detected and classified. The number of Porechop demultiplexed reads ranged from 90 to 11,974 for Flongle and from 614 to 73,261 for MinION flow cell.As per its lower number of provided pores, Flongle sequencing predictably produced fewer reads which consequently resulted in lower mean quality compared to a MinION flow cell. However, our study confirmed that the Flonglle flow cell can be cost-effective for the rapid whole-genome long-read sequencing of HPAI in clinical samples during an outbreak or mass screening.

FTA Card Use as a Sample Collection Method to Type Avibacterium paragallinarum

Rachel Jude
University of California, Davis

Avibacterium paragallinarum, the bacteria responsible for infectious coryza in chickens, is an economically significant agent due to its effects on growth performance and egg production. Outbreaks of A. paragallinarum occur worldwide and FTA card technology allows for safe transport of samples to be assessed via molecular serovar typing and to construct a complete epidemiological picture. Here, we investigated the quality of sequences obtained by the Sanger method from various sample types (cultures, DNA extracts, tissue impressions, and vaccines) applied into FTA cards. DNA was isolated from the FTA cards and a 1,224 bp portion of the Hmtp210 gene was amplified and sequenced. Obtained reads were assembled and analyzed for quality using several parameters (confidence mean, expected error, and phred scores) as well as for identity to reference sequences available on GenBank. Overall, sequences from DNA extracts and cultures applied to FTA cards were comparable in terms of sequence quality and identity to A. paragallinarum and can therefore be equally endorsed samples for transport via FTA cards. The ability to utilize FTA-based sampling for molecular serotyping of A. paragallinarum aids in overcoming sampling and transportation constraints that may prevent a full repertoire of serovars to be analyzed.

Analysis of diagnoses for broiler chicken flocks in Korea between 2017 and 2021

chung-hyun Kim
QIA

Korean chicken meat is consumed as fried or samgyetang, which is cooked whole rather than separated meat. According to Korean's unique food culture, Korean broilers are slaughtered at 1.5kg, approximate 30~35days of age. Considering the short-term breeding period different from other countries, we analyzed of necropsy report over a 5-y period (2017-2021) from diagnostic laboratories in the Korea by KAHIS (Korea Animal Health Integrated System); 2,834 cases were collected. The primary cause of mortality was categorized as viral/bacterial/fungal/parasitic infection, toxic/nutritional disease and undetermined. Bacterial infection was the most common primary diagnosis and involved with 62.0% of all

broiler chicken inspected, followed by viral (20.0%), parasitic (2.2%), nutritional (0.4%), fugal (0.2%) disease. In bacterial diseases (n=1,737), colibacillosis was most common bacterial infection (47.7%) and followed by salmonellosis (42.5%), Enterococcus spp (3.0%), Clostridium spp (1.1%) and staphylococcus spp (1.0%). In viral diseases (n=568), fowl adeno virus (FAdV) was most commonly detected virus (54.8%), followed by infectious bronchitis virus (IB, 19.9%), infectious bursal disease (IBD, 11.3%), chicken infectious anemia (CAV, 3.9%), and avian encephalomyelitis (AE, 1.9%). Additionally, Coccidiosis (91.8%) was most common in parasitic diseases. This results regarding prevalence of infectious diseases can help flock owners to educate for disease prevention and biosecurity practices and veterinarians to diagnosis.

Identification of the causative agent in broiler chicken showing neurological symptoms

Hye-Ryoung Kim
APQA

The 32-day-old broiler chickens showed neurological symptoms like walking with a limp, lying down and head shaking. About 1% of chickens died or were culled. Five carcasses from a farm located in northwestern South Korea were submitted to the Avian Disease Division of the Animal and Plant Quarantine Agency (APQA) for disease diagnosis. Necropsy, bacteriological culture, virus detection using RT-PCR, pathological examination, and electron microscopy were inspected using an APQA diagnostic protocol on the basis of clinical manifestation and the presence of gross lesions. In gross lesions, severe epi-carditis and epi-hepatitis were observed. Microscopic lesions were observed in the cerebrum and in the granular layer of the cerebellum, consisting of multifocal perivascular cuffing and purulent necrosis in the cerebrum and severe meningitis with heterophil and lymphocyte infiltration.Staphylococcus spp. were recognized in the liver and heart using bacteriological culture. The RT-PCR examinations to detect avian encephalomyelitis virus was performed, and the result was negative. To explore further etiology, bacterial and viral metagenomic analysis were performed using brain samples.

Infectious bronchitis molecular surveillance in layer farms in Southern California

Evelin Saenz
UCDAVIS

Infectious bronchitis virus (IBV) represents an important economic burden for commercial egg layers causing acute respiratory and urogenital disease. Failure to peak is one of the most common productive issues associated with laying hens affected by some IBV genotypes. Even though this condition might reflect multiple infections, IBV is usually part of this condition. In order to assess IBV involvement, affected farms in Southern California were invited to an IBV surveillance strategy. Tracheas, kidneys and cecal tonsils were collected at different ages according to their specific vaccination program (chicks, pullets, peak of production and late production). The IBV genetic material was extracted, and RT-PCR was performed amplifying a portion of ~800bp of the S1 gene hypervariable region. RT-PCR products were sent for sequencing for genotype determination. Detected sequences will be analyzed and compared in phylogenetic analyses including local and suspected variants and vaccine genotypes used in this geographical region

Enteric Health

Feed quality assessment with the use of glucose oxidase vs antibiotics

Josue Sanchez
excelling SA de CV

The objetive of this study was to evaluate the stool quality of the treatment (Tx) glucosa oxidase 3,000 IU (GOX) supplemented versus control treatment. The test was conducted on a laying hen production farm (Hy line w80), elevated, natural weather, automatic collection and feeding, divided into two modules with 3 houses, each houses 200,000 birds. Fecal collection slides were made with dimensions Length: 50 cm, width:40 cm, were fixed to the cage at a distance of approximately 10 cm. Two treatments were formed: Treatment 1 supplemented with 100 ppm of GOX (Module 1) and treatment 2 supplemented with 500 ppm of antibiotic (Module 2). Nine replicates were out with 5 animals per treatment. Slides were seeded at the same time in both groups, 24 hours later the following were collected. The evaluation was carried out through the observation of characteristic such as coloring, form, size and apparent humidity; the corresponding color was assigned to the characteristic to the Brugere-Picoux to stool abnormalities and accounted. A completely randomized design was used, the data obtained were subjected to a Chi-square analysis using SAS linear models. The results indicate that the treatments are statistically different ($p < 0.05$). This suggests that the implementation of alternatives to the use of antibiotics such as glucose oxidase improves the intestinal integrity of the bird and is reflected in the quality of the feces, indicative of better intestinal immunity in birds.Key words: Intestinal integrity, glucose oxidase, stool quality, diagnostic tool.

Ileal mucosal microbiota in commercial turkey poults

John Ngunjiri
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Gut microbiota are a promising target for development of intervention strategies to promote health and production performance of commercial turkeys. Digesta-associated microbiota (DAM) and mucosa-associated microbiota (MAM) may have different community structures and shift independently in response to changes in the gut environment. Until now, turkey studies have used DAM or fecal microbiota as a proxy for gut microbiota and MAM remain uninvestigated. Here, we used samples of ileal digesta, homogenate, wash, and mucosa scraping to compare DAM and MAM communities in 4- and 9-weeks-old commercial turkey poults through 16S rRNA gene amplicon analysis. We have applied a relative efficiency statistical method first employed by L.R. Taylor in agricultural entomology and found that microbiota recovered through digesta and gut tissue homogenate sampling methods are highly similar. Furthermore, wash and mucosa scraping methods were highly efficient at sampling ileal MAM. Finally, we have provided a comprehensive account of potential MAM of the turkey ileum, which could be targeted for improving turkey health and production performance.

Effects of different methionine to cysteine ratios on the gut health, oxidative status, gene expression of nutrient transporters, and tight junction proteins of broiler chickens challenged with Eimeria spp

G Liu
Univeristy of Georgia

ILT/Marek's Disease

Longitudinal Study of Infectious Laryngotracheitis Virus (ILTV) in Commercial Laying Farms from Sao Paulo State – Brazil

Renato Luciano
Biological Institute - Advanced Center of Poultry Research

Different vaccine programs were established in the two quarantine regions in the State of Sao

Paulo (Bastos and Guatapara) after the first detection of infectious laryngotracheitis (ILTV). Live attenuated vaccines (CEO and TCO) were firstly used and replaced by recombinant vaccines. Our longitudinal study collected 578 oropharyngeal swab pool samples from commercial layer chickens located in Bastos (n=364) and Guatapara (n=214), from 2010 to 2018, for ILTV detection. Samples were analyzed by PCR; DNA sequencing (Sanger) and next-generation sequencing. ILTV was detected in 11.85% and 12.6% of tested samples from Bastos in 2013 and 2018, respectively. The Guatapara region had the highest detection rate (60.5%) in 2010. After 2013, the detection rate from tested samples of the Guatapara region ranged from 12.2% to 21.7%. Phylogenetic analysis of Bastos sequences grouped with CEO vaccine and sequences previously reported in the area with a nucleotide identity of 99% (KJ028222, KJ028224). Sequences from Guatapara were grouped with virulent ILTV previously detected in 2015 (MF678664), with 100% of nucleotide identity. The partial genome sequence of one sample (IB8098) collected in Guatapara (2010), with a length of 152,985 nucleotides had 4,755 reads and coverage of 98.2%. The IB8098 sequence was grouped with virulent ILTV strains, such as Russian (MF405079) with a high (99%) nucleotide identity. The vaccination programs in the quarantine regions decreased the virus circulation; however, the ILTV detection was still reported when using the recombinant vaccine. Continuous monitoring of the area will be essential to evaluate the vaccination measures placed.

Characterization of the Backyard (Non-Commercial, Non-Quota) Poultry Population in Alberta, Canada, and the Submission Level Prevalence of Infectious Laryngotracheitis Within this Sector

Heather Van Esch
Government of Alberta, Ministry of Agriculture, Forestry and Rural Economic Development

The increasing popularity of non-commercial, or “backyard” poultry keeping in Alberta, Canada, and the endemic nature of the provincially reportable disease, Infectious Laryngotracheitis (ILT), within this population have raised questions as to whether these flocks may pose a disease risk to commercial poultry operations in the province. However, the scarcity of baseline information about these flocks makes it difficult to draw any conclusions in this regard.For this study, results from a voluntary online questionnaire available on Alberta backyard poultry social media sites between October 1, 2019, and March 31, 2020, and provincial government disease surveillance and traceability program data from a 5-year period ending on December 31, were used to estimate the size of the Alberta backyard poultry population, describe the characteristics of these flocks, and determine the submission level prevalence of ILT within this sector.Questionnaire respondents were found to be primarily middle-aged, well-educated females from acreage locations. Most had been keeping poultry for less than 5 years, and the majority had less than 50 birds. The non-participation rate in the “mandatory” government traceability program (Premises Identification, or PID program) was 27%. There were estimated to be over 10,300 backyard flocks within Alberta in 2020, representing 128,350 – 409,000 laying birds. The submission level prevalences of the three most common diseases diagnosed in the government disease surveillance program were Marek’s Disease (18.6%), Mycoplasmosis (11.7%) and ILT (11.1%). The results of this study can be used as the basis of future research for this underserved and underrepresented population.

Evaluation of the impact of injector device on Marek's disease vaccine delivery by subcutaneous route

Andrea Delvecchio
Boehringer Ingelheim AH

Marek's disease (MD) has been recorded for decades as one of the major diseases of poultry. Vaccination represents a key factor to prevent and control the incidence of the disease worldwide. Over the years, vaccination programs against MD have evolved and different types of cell associated MD vaccines are nowadays applied alone or in combination to improve control of field strains. Vaccine handling and application at the hatchery is a sensitive step to ensure a proper MD protection in the field.In our study we evaluated the parameters that could negatively impact the MD vaccine viability and consequently the proper vaccine intake. For that purpose, a commercial injector device as commonly used at hatcheries for subcutaneous administration of MD vaccine, was evaluated. Several conditions, such as the pressure of injection and the diameter of needle were tested. In a first set of in vitro trials the impact of the device on MD vaccine viability (HVT vaccine) was assessed by virus titration comparing the titers before and after passing through the injection system. Additionally, cell viability of the MD vaccine was evaluated by flow cytometry.The results showed that the device itself, alone or with needles of different diameters did not have any impact on the titers of the HVT vaccine. Nevertheless, a not significant impact was recognizable on cell viability: the smaller the diameter of the needle used the stronger was the effect on cell integrity. In addition, in vivo evaluation of the vaccine intake was performed.In conclusion, in this study we evaluated some of the factors that could negatively affect the MD vaccine delivery at the hatchery. The injector used in the trials proved to be suitable for MD vaccine administration. Further studies are needed to explore other factors that could impact the MD vaccination.

Use of a Novel Medium to Grow Chicken Embryo Fibroblasts that Increases the Yield of Marek's Disease Vaccines

Isabel Gimeno
North Carolina State University

The cornerstone in the control of Marek's disease (MD) is vaccination. MD vaccines are cell-associated and grow in chicken embryo fibroblasts (CEF). One of the major limitations when growing MD vaccines is that infected cells tend to die and it is critical to trypsinize cells when the largest number of infected cells can be retrieved. Infectivity rate, however, is usually low and that is one of the factors why there is great variability in the dose administered to chickens even when using the same vaccine vial. Also, the low infectivity rate limits the amount of vaccine doses that could be added into a vial and increases vaccine production costs. In the present study we have evaluated the growth of HVT, one of the MD vaccines most used, in a novel medium (Diploid Growth Serum Reduced Medium). This medium allows CEF to grow with limited amount of calf serum or even without serum. In our study, the best results to grow HVT were achieved when supplementing the medium with 1% calf serum. With this medium, CEF tend to become multilayer and HVT grows extensively in the plates for a longer period of time compared to using conventional media (modified Leibovitz-McCoy). Yield of HVT in the Diploid Growth Serum Reduced Medium was at least twice than when using conventional media and infectivity rate increased as well. The impact of these results on the production of MD vaccines will be discussed.

Immunology

Assessment of Seroconversion In Commercial Layers During The Rearing Period Using Three

Different Elisa Kits After Administration Of An Immune-Complex Vaccine (Novamune®) At One-Day-Old.

Oscar Sanabria
Ceva

Assessment of Seroconversion In Commercial Layers During The Rearing Period Using Three Different Elisa Kits After Administration Of An Immune-Complex Vaccine (Novamune®) At One-Day-Old.Mauricio Sanabria1, Ramiro Delgado2, Hernando Guzmán2, Paulina Zuluaga3, Vanessa Flórez3, Jaime Sarabia4, Roberto Soares4, Rafael Forero5, Andrés Gil6, Juan Carlos López7Ceva Animal Health1 Colombia, Nutriavicola2 Colombia, Quinolab3 Colombia,Ceva Animal Health4 Francia, IdVet5 Francia, LMV SAS6 Colombia, Consultor estadistico7 Colombia Corresponding author: mauricio.sanabria@ceva.comThe aim of this study was to investigate the effect of a novel Immune-complex vaccine on immune system response in commercial layers. The presence of infectious bursal disease virus (IBDV) antibody was assayed using three different indirect Elisa tests, namely, Idexx, Biocheck and IdVet, following the manufacturer's instruction strictly. A commercial layer flock was vaccinated at one-day-old using Novamune®, an immune-complex vaccine using the subcutaneous route. During the rearing period, serum samples were taken on days 1, 14, 21, 28, 35, 42, 56 and 70. Overall, during the different sampling days, the lower mean titers and higher variability were obtained using the kit Idexx. In contrast to, the higher mean titer was determined when the Biocheck kit was reported as. In the case of the Biocheck kit, after 21 days, all the serum were reported as positive; when Idvet or Idexx kits were used 100% of positive serums were detected up to the fourth week of life.The Pearson Linear Correlation Coefficient was executed using the software R to determine the linear relationship between the three different kits. The correlation between Biocheck and Idexx was 0.783*, in case of IdVet and Idexx a similar value was obtained (0.074*), finally when the linear relationship was done among Biocheck and Idvet the Pearson return value correlation was 0.969***, which means a strong linear association between these two Elisa kits.In conclusion, for IBDV antibodies level, there is a strong relationship when the Biocheck or IdVet kits are used, in contrast, a low grade of correlation was obtained in the case of Idexx with the other two kits.

Comparison of Transcriptional Analysis, ELISA, and Biological Assays of Avian Type I IFN

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The innate immune system, especially through interferons (IFNs), serves as one of the first layers of host defense against viral pathogens including influenza and Newcastle disease viruses. Real-time quantitative PCR has been extensively used for transcriptional analysis of cytokine gene expression. However, the gene expression approach does not always correlate with protein-based or functional assays. We previously optimized a biological type I IFN assay and utilized the assay to identify high interferon inducing influenza viruses to select vaccine candidates. The assay was applied to determine the level of IFN induction upon influenza virus infection in quail fibroblast cells and compared with gene expression data using quantitative PCR. The results clearly showed that gene expression data does not always correlate with biological assay data depending on the kinetics of virus replication which has to do with virus and host interaction. Although biological type I IFN assay is highly useful, the assay is rather time consuming. In this presentation, we will discuss the value of commercial ELISA in quantifying IFN level in comparison to biological IFN assay.

Immunometabolic regulation of CpG-ODN-induced antimicrobial trained immunity in chickens

Iresha Subhasinghe
University Of Saskatchewan

Immunometabolic regulation of CpG-ODN-induced antimicrobial trained immunity in chickensIresha Subhasinghe A, Khawaja Ashfaque Ahmed A, Hemlata Gautam A , Arzhang Shayeganmehr A, Shelly Popowich A, Betty Chow-Lockerbie A & Susantha Gomis A*A Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, S7N 5B4, Canada*Corresponding author: Email: susantha.gomis@usask.caAbstractSynthetic DNA containing Cytosine phosphodiester Guanine motifs (CpG-ODN) acts as a pathogen-associated molecular pattern (PAMP) orchestrating antimicrobial immunity against bacterial pathogens in chickens. We have previously reported that CpG-ODN-induced antimicrobial state involves immune activation and the enrichment of immunological niches and metabolic changes in chickens. Here, we thought to investigate CpG-ODN induced immune-metabolic interactions. In the first set of experiments, a week-old broilers chicks were administered CpG-ODN or saline via the intramuscular route. In the second set of experiments, birds were in-ovo injected with either CpG-ODN or saline, followed by Oralvaccine after hatch. The peripheral blood was collected longitudinally at several time points to assess antimicrobial activities of chicken heterophils using flow cytometry-based methods, and the metabolic status of immune cells was evaluated in real-time using Seahorse XFp metabolic analyzer. Our data showed a significantly (P 0.05) higher in CpG-ODN groups, indicating better metabolic fitness of the immune cells in CpG-ODN groups. To the best of our knowledge, this study reports for the first time the role of the CpG-ODN-mediated immunometabolic regulation in the orchestration of protective antimicrobial immunity in chickens.

The impact of antibiotic growth promoters on intestinal health during a subclinical necrotic enteritis challenge in broilers

Shailes Bhattarai
University of Georgia

Infectious Bronchitis Virus

Phylogenetic Analysis of Infectious Bronchitis Virus Circulating in Colombia From 2015 to 2021

Oscar Sanabria
Ceva

Phylogenetic Analysis of Infectious Bronchitis Virus Circulating in Colombia From 2015 to 2021Leonardo Alvarado1, Mauricio Sanabria1, Carlos Acevedo1, Sjaak de Wit2, Luiz Sesti3, Jaime Sarabia4, Nestor Mossos5Ceva Animal Health 1Colombia, 3Latin America, 4France2GD (Animal Health Services) Deventer, The Netherlands, 5Consultor Científico, Colombiacorresponding author: mauricio.sanabria@ceva.com SummaryInfectious bronchitis (IB) is a widespread disease caused by the infectious bronchitis virus (IBV) which is an epitheliotropic germ that causes acute and severe disease in birds. Molecular and epidemiological studies showed that IBV circulating in Colombia belong to various genotypes although only Massachusetts strains-containing live vaccines are authorized. Between 2015 to 2021, twenty-seven samples collected from flocks suspicious of IBV infection across the country were submitted to Royal-GD using FTA-imprints. For

each sample, in the case the RT-PCR was IBV positive, sequencing was performed for genotyping. Primers used were XCE1-XCE3 and, subsequently, computer analysis was done to calculate the genetic relatedness to other known S1 sequences using MEGA-X software.Twenty-six strains were genotyped by the Sanger method. A total of six different genotypes of IBV were detected. Of those, only three were Mass-like vaccine strains. The other 23 strains were characterized as field strains of variant genotypes Q1, 793B, YEM/L-2865/2005, PRT/L-898/2004 or K46/10(JF804676). The YEM/L-2865/2005 was the predominant genotype, reported ten times. After that Q1 and 4/91-793B are the most prevalent strains detected during this period.The high number of variant IBV strains (5 genotypes) detected suggests the use of only Massachusetts vaccines will be insufficient to induce appropriate levels of protection against variant strains. It's quite well known that field and experimental results clearly indicate that using a single serotype of IBV vaccine doesn't afford adequate protection from heterologous challenge. Furthermore, using the multi-monovalent strategy with Massachusetts vaccine-strains may not provide protection against the variant viruses and other variant serotypes may be observed in face of vaccination.In conclusion, several variant genotypes of IBV are present in Colombia and are causing economical losses, despite the intensive use of live and killed Massachusetts vaccines clearly indicating that the strategy has not been sufficient to control the field IBV challenge.

Isolation, Genotyping and Evaluation of Pathogenicity of Different Egyptian Infectious Bronchitis Viruses

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Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV), is an extremely contagious disease of chickens leading to tremendous economic losses to the poultry industry worldwide. Different genotypes and pathotypes of IBV are cocirculating in Egypt, resulting in different clinical pictures manifested as respiratory, renal and/or reproductive. In this study, IBV was detected as a cause of nephropathy in eight broiler farms located in seven governorates in Egypt using RT-PCR. Partial S1 gene sequence and phylogenetic analyses revealed that three strains were related to IBV strain 6/82 and five strains were related to the Israeli strain IBV IS/1494/06. Two IBV strains, namely, IBV/CU-2 and IBV/CU-4, representing 6/82 and IS/1494/06, respectively, were then isolated in specific pathogen free (SPF) embryonated eggs and their pathogenicity were further evaluated in one-day-old SPF chicks. Our results showed that both isolated IBV strains needed seven serial passages for egg adaptation to induce curling and dwarfism of the chicken embryos. Moreover, infected embryos showed deposition of ureates in mesonephrous emphasizing the nephro-pathogenicity of the isolated strains. After intranasal inoculation in one-day-old SPF chicks, IBV/CU-2 and IBV/CU-4 induced varying degrees of coughing, sneezing, tracheal rales, opened mouth breathing and wet droppings with mean clinical scores of 2.3 and 2.5; (clinical scores range from 0; normal to 3; severe), and mortality of 20% and 30%, respectively. Infected chicks also showed gross and microscopic pathological changes in tracheae and kidneys. These results indicate that both IBV/CU-2 and IBV/CU-4 are highly pathogenic to chickens possessing respiratory and renal tropism.

Homologous and heterologous live-attenuated vaccines strategy to reduce infectious bronchitis virus transmission

Robert Beckstead
Ceva Animal Health

Infectious bronchitis virus (IBV) is a highly contagious respiratory gammacoronavirus in chickens that is associated with decreased egg production and nephritis. Homologous and heterologous live-attenuated vaccines are used to control IBV. One additional disease challenge is related to the variety of circulating field viruses. Recently, DMV1639 variant IBV became a critical threat to the US poultry industry. The aim of this study was to test if a live vGA08 and Mass vaccination in broilers would control disease transmission when half of the vaccinated birds were challenged with pathogenic GA08, Mass41 or DMV1639 isolate at 28 days post vaccination (dpv). Non-vaccinated, challenged birds were used as positive controls. Non-direct challenged birds were added to the flock 1 day post challenge (dpc). Vaccine takes at 5dpv and viral shedding at 2-14dpc were determined by RT-qPCR of choanal-cleft swabs from individual birds. Vaccine takes were 100% for vGA08 and 85% for Mass. All non-vaccinated direct challenge birds and non-vaccinated, non-challenged contact birds were positive for virus starting at 2dpc. Low levels of IB RNA were detected in 30% of the GA08 (Ct>30), 70% of the Mass41 (Ct>34), and 40% of the DMV1639 (Ct>35) vaccinated direct-challenged birds. In the vaccinated contact-challenged birds, 10% of the GA08 (Ct>38), 70% of the Mass (Ct>32), and 0% of the DMV1639 were positive for IB RNA. No virus was detected in the vaccinated contact-challenged birds after 7dpc (GA08) and 9dpc (Mass). Vaccination with vGA08 and Mass controlled viral shedding in both the GA08 and DMV1639 challenged and contact birds. Although 70% of the Mass41 challenged and contact birds were positive for IBV, there was a 3-log reduction when compared to the non-vaccinated Mass41 challenged control birds. This data supports the reduction of IB RNA detection and transmission when heterologous vaccines are utilized in front of a DMV1639 challenge.

Isolation of Infectious Bronchitis virus variant strain from commercial layer birds showing cystic oviduct in India

Namdeo Bulbule
Venkateshwara Hatcheries Pvt Ltd

Infectious bronchitis virus (IBV) is a major pathogen of poultry affecting respiratory system. It may affect urinary tract and reproductive system and causes kidney damage, drop in egg production and abnormal egg shell. In India, emergence of nephropathogenic IBV was first reported in 2005 and since then several outbreaks of Infectious Bronchitis involving nephropathogenic forms were reported. In our study we have isolated variant IBV from the commercial layer flocks with the history of 10-20% non layers and the flock not attaining peak egg production. On post mortem examination birds revealed cystic left oviduct. Attempts were made to isolate the pathogens by various methods. Sentinel experiment was carried out by placing 200, day-old specific pathogen free (SPF) chicks in affected farm where brooding and growing was done for affected flock. Serum and tissue samples were collected from sentinel birds at different intervals post exposure and analyzed by serology, virus isolation and histopathology. Trachea, kidney and cecal tonsils were collected from 3 to 7 days post exposed SPF chicks, found positive for IBV by qRT-PCR. Cecal tonsils and Kidney samples showed higher concentration of IBV as compared to trachea. IBV was isolated from the same samples and confirmed by qRT-PCR and sequencing of spike S1 gene. To know the pathogenicity and its role in induction of cystic oviduct, virus titration was carried out and 0.2ml allantoic fluid (105.8 EID50 virus titer/ml) was inoculated in day-old SPF chicks by intra nasal/ocular route. Mortality was observed on 5 days post inoculation and kidney lesions were most prominent suggesting the

nephropathgenic character/nature of the virus. At 6 to 10 weeks post inoculation, some birds showed cystic left oviduct. All the survived birds were sacrificed at 28 wks post inoculation and experiment was terminated. Sequential histopathological examination indicated that the IBV infection directly damage the immature oviduct resulting in cystic oviduct. Antigenic drift in S1 gene nucleotide/amino acid sequences leads to the emergence of IBV variant strains affecting kidney and oviduct.

Real World Evidence: Economic Assessment of the Impact of Q1-like Infectious Bronchitis Variant in a Broiler Productive Zone in Peru

Claudia Carranza
Ceva Animal Health

Real World Evidence: Economic Assessment of the Impact of Q1-like Infectious Bronchitis Variant in a Broiler Productive Zone in PeruCarranza Claudia1, Lecoupeur Mathilde2, Cotta Higor2, Sesti Luiz3Veterinary Services - Ceva Animal Health, Peru1, Real World Evidence Unit2, Veterinary Services - Ceva Latin America3Infectious Bronchitis Virus (IBV) is known to cause mild to severe respiratory signs in poultry. In Peru, IBV has been present since the late 1960s, and IBV variants were detected in 2009 in flocks with severe respiratory and renal lesions. Epidemiological studies have shown the presence of a diversity of variants in the Peruvian territory, with the Q1-like being the predominant one. In Peru, vaccination against IBV is performed in 100% of hatched broilers. However, the only approved serotype is Massachusetts, which confers limited protection against the real Peruvian IBV field challenge. Birds vaccinated with Mass-type vaccines that experience field challenges with variant strains of IBV may present a variety of clinical signs or develop a subclinical infection. This subclinical infection underestimates the real impact of IBV because in these challenged flocks (that generally express lower performance), IBV usually stays undetected. Finally impacting the economic benefit of the company. So, the objective of this study was to monitor IBV in a poultry production zone and to compare the performance of challenged vs. non-challenged flocks to determine the economic impact of IBV variants in Peru. The monitoring of sanitary conditions was performed with serology at slaughter age, qRT-PCR of cloacal swabs at 35 days of age, and sequencing of positive samples. For the statistical data analysis and data visualization, the Python programming language coupled with Numpy/Scipy modules was used. The “Ceva Economical Calculator” software was used to the assessment of the overall economic impact. Our results show that IBV variants economically impact broiler production in Peru.

Data Management as a Strategy for Serological Monitoring of Infectious Bronchitis in broiler farms

Jose Perez
SPM, NCSU

Data Management as a Strategy for Serological Monitoring of Infectious Bronchitis in broiler farms Pérez, José1 Sanitary Manager, Grupo Santa Elena, Peru1Serologic monitoring in the poultry industry is routinely performed. Blood samples are collected mainly at slaughter age and new serologic sanitary data is created daily from its analysis. Indicators as Arithmetic mean, Geometric mean, minimum, maximum and coefficient variation are discussed as part of the monitoring strategy of every poultry production company. However, with the increased availability of Data Management software we were able to apply a new monitoring strategy for Infectious Bronchitis in broiler farms. Therefore, the objective of this work is to describe a new approach regarding the analysis of serological data, using the individual titers obtained during a period of 12 months. For the statistical data analysis and data visualization, the Python

programming language coupled with Numpy/Scipy modules was used.

A broad protection spectrum against relevant strains can be achieved by the combination of Mass-type and BR-I-type infectious bronchitis vaccine strains

Jorge Chacon
Ceva Animal Health

The absence of homologous vaccine strains that can be used against an increasing number of variants of Infectious Bronchitis (IB) virus circulating around the world led to evaluate combinations of available IB vaccine strains. Protection conferred by live BR-I-type (GI-11) and Mass-type (GI-1) vaccine strains against 793B (GI-13), Q1 (GI-16) and Variant 2 (GI-23) serotypes was tested. Vaccines were applied simultaneously to one-day-old SPF chicks oculo-nasally and challenges were performed 3 (Var-2) or 4 (Q1, 793B) weeks post-vaccination. The vaccine strain combination showed to be safe for chickens because no relevant clinical signs, mild gross and microscopic lesions were recorded. Efficacy was measured based on protection against respiratory signs, ciliostasis, microscopic lesions in trachea and kidney, and challenge virus replication at 5 days post-challenge. The vaccine combination conferred 85, 93, and 100% of protection against ciliostasis caused by the 793B, Var-2-type and Q1-type challenge strains, respectively. Vaccination induced a strong and significant suppression of challenge virus replication (3.7, 4.2 and 5.7 log10 reduction of mean total 793B, var-2 and Q1 IBV RNA amount in the trachea, respectively, compared to the corresponding non-vaccinated challenged control groups). In case of Var-2 challenge, kidney protection and cloacal shedding was also assessed based on IBV Var-2 selective measurement by RT-real-time PCR, which showed significant effect of vaccination. The study demonstrated that BR-I and Mass-type vaccine strains used in combination are useful tools for control of high-prevalence and worldwide circulating variant IBV strains.

Persistence and intensity of Infectious Bronchitis Virus Vaccine Strain replication and its association with safety aspects

Jorge Chacon
Ceva Animal Health

Live vaccines are essential tools for Infectious Bronchitis (IB) control. Because many times there are no optimal poultry farming conditions, severe post vaccination reactions can be observed following IB vaccination. The aim of this trial was to compare two live IB vaccine viruses based on the replication kinetics in the trachea and the severity of macro and microscopic changes after vaccine application. Two BR-I (GI-11) live vaccines were oculonasally applied to two groups of 1-day-old-SPF-chickens (Vaccines 1 and 2). Clinical signs, ciliostasis, microscopic lesions in trachea and kidneys were evaluated and scored at 7, 10 and 29 days post vaccination (dpv). Both vaccine viruses showed different attenuation levels because they reached different scores. Chickens receiving “vaccine 2” presented higher clinical and trachea microscopic lesion scores at 10 dpv. Full ciliostasis was observed in case of ‘vaccine 2’ in 100 and 90% of chickens at 7 and 10 dpv, respectively, whereas after ‘vaccine 1’ it was seen in 70 and 20%, respectively. Vaccine virus causing more severe and persistent lesions in respiratory and renal tract (vaccine 2) had higher virus replication rate (2.02 log10 higher amount of IBV RNA at 10 dpv) and longer virus persistence (virus detected in 50% of vaccinated chickens up to 29 dpv). No vaccine virus was detected in chickens inoculated with “vaccine 1” at 29 dpv in respiratory tissues. This work showed that intensity and persistence of vaccine virus replication can be used as indicators of the difference in safety margin of IB live vaccine viruses.

Complete Genome Analysis of Two Live Attenuated Infectious Bronchitis Virus Vaccine Candidates Provide Insight Into the Attenuation Mechanism

Yun Jeong Choi
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Infectious bronchitis viruses (IBV) cause highly contagious and acute multi-systemic (respiratory, reproductive, and renal) disease in chickens. QX-like (GI-19 lineage) IBV viruses have spread all around Asia, Europe, and Africa. Since its first introduction in the early 2000s in South Korea, QX-like IBVs became endemic in the South Korean poultry industry. Classical attenuation method of IBV is serially blind passaging the virus in embryonated chicken eggs until viruses do not show clinical signs when infected in chickens. However, blind passaging as vaccine development of IBV is costly and time-consuming, requiring more than 100 passages, resulting in delayed response to the rapidly evolving pathogen. Previous studies have shown that blind passaging combined with heat treatment expedited vaccine development of IBV vaccines.Two different IBV strains (K1277/03 and QX1830029) in the GI-19 lineages were serially passaged combined with the heat treatment method in embryonated chicken eggs until they were apathogenic in chickens. The complete genome sequences of the novel IBV vaccine candidates were obtained by NGS methods using the Illumina platform. K1277/03 HP90 acquired a total of 17 SNPs resulting in 9 nonsynonymous mutations and 196bp of deletion of 4b and 4c accessory proteins. QX1830029 HP60 acquired 10 SNPs resulting in 7 nonsynonymous mutations and 42bp of deletion 6b accessory proteins. Both vaccine candidates possessed truncation of 27bp at the cytoplasmic domain of S2 of the spike protein. Complete genome sequencing of IBV vaccine candidates revealed potential hotspots of mutations contributing to the attenuation of the viruses.

Genome Sequence Variations of Infectious Bronchitis Virus Serotypes from Commercial Broilers in Mexico

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USDA-ARS

Novel variants of infectious bronchitis viruses (IBV) emerge continually despite routine vaccinations. Here, we report sequence variations of IBVs identified by random untargeted next generation sequencing (NGS) of 3 vaccine and 132 field samples collected on FTA cards from commercial broilers in Mexico in 2019-2021. Paired-ended DNA sequencing libraries prepared by random priming-mediated SISPA/Nextera Flex protocols from total RNAs (pre-treated with an in-house RNaseH host rRNA depletion) were sequenced using Illumina MiSeq Reagent Kit v3, followed by de novo assembly using MIRA in Galaxy and annotation in Geneious Prime platforms. Forty-four samples contained IBV RNA, out of which 21 field and the 3 vaccine samples produced complete genome sequences (27,022 - 27,805 bases in length) belonging to lineages GI-13 (793B; n=10), GI-1 (Mass-type; n=7), GI-9 (Ark99; n=5), and GI-3 (Holte/Iowa; n=1). The remaining 21 samples produced partial genome (n=6), complete and partial S-gene (n=2 and 13, respectively) sequences. Based on the S-gene, two and one of the vaccine sequences belonged to the Mass-type and 793B serotypes, with 96.9-98.1% and 94.3-94.6% nucleotide identity to the field sequences in respective lineages. Preliminary recombination analysis of the S-gene indicated potential recombination events in six of the 23 sequences and in other serotypes within the same lineages. Analysis of insertions/deletions and mutation in the deduced amino acid sequences of the hypervariable regions 1 and 2 (HVR1 and HVR2) of the S1 revealed 3- and 2-amino acid deletions (residues 59-61 and 61-62) in the HVR1 of 2 sequences in lineages GI-1 and GI-3, respectively, when compared to serotypes

within the same lineages. These data suggest circulation of novel IBVs in Mexican commercial flocks, possibly generated from mutations and inter-strain recombination. Robust active surveillance of IBVs, and review of the current vaccines using the IBV strains currently circulating in Mexico and the region cannot be overemphasized.

Surveillance of Infectious bronchitis virus occurred in Korea, 2021

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Infectious bronchitis virus (IBV) is a highly contagious and acute respiratory disease that causes economical loss in poultry industry. Infected chickens damaged to upper respiratory tract, kidney and reproductive organ may exhibit respiratory signs, nephritis and decreased egg quality and production. In addition, affected chickens are susceptible to secondary bacterial infection such as Escherichia coli. Vaccination is important strategy to prevent outbreak of IBV. Because cross-protection between different serotypes is limited, it is difficult to control of IBV. Thus, continuously investigation of infectious bronchitis virus needs to apply appropriate vaccines.S1 subunit of spike glycoprotein contains receptor-binding domain and affects to generation of neutralizing antibodies. Also, S1 subunit can be utilized to determining of serotype and genotype.We isolate 27 cases of IBV in Korea, 2021 and sequenced of S1 subunit gene to classification of IBV genotype. Among isolates, two of twenty-seven IBV were classified as GI-15 and rest as GI-19. However, one of IBV classified as GI-15 is suspected to occur recombination of GI-15 and GI-19. It is needed to prolonged monitor for the recombination virus whether will be widespread or not in Korea and further study for properties such as cross-protection, pathogenicity.

Detection of GI-13 of infectious bronchitis virus in layer chickens in Brazil

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The Brazilian infectious bronchitis virus (IBV) lineage, the GI-11, has been circulating in the country since 1975. Two studies detected other genetic IBV lineages (GI-12 and GI-13) in the country in 2008. The present study aimed to investigate the pathogens causing clinical respiratory signs in a chicken layer farm. In July of 2021, a layer flock with 250 multiage (25 to 70 weeks-old) chickens showed depression, tracheal rales nasal discharge, and coughing, drop in egg production, ruffled feathers, kidney congestion, swelling of the facial skin and the eyelids, with accumulated mortality of 8%. Swabs (oropharyngeal and cloacal), kidney, ovary, liver, and sera were collected from five sick birds ten days after the onset of clinical signs. Sera were tested for infectious bronchitis, Mycoplasma synoviae (MS), Mycoplasma gallisepticum (MG) using IDEXX indirect kits. Tissue and swab samples were screened for respiratory viruses (avian influenza virus, Newcastle disease virus, IBV, and metapneumovirus) by real-time RT-PCR, ELISA titers for IBV, MG, and MS were 3886, 10812, and 12537, respectively. Swabs and tissue samples were negative for all tested viruses, but IBV was detected in the cloacal swab sample (Ct=24). Phylogenetic analysis based on the S1 gene grouped the detected virus into GI-13 lineage, with nucleotide identity of 97.2% when compared to the previous GI-13 sequence detected in Brazil in 2008 (FJ791272). Our study suggests the GI-13 has been circulating in Brazil with other respiratory pathogens. Continuous surveillance of respiratory viruses in poultry farms should be carried out to monitor the emergence of pathogens.

Development of a live attenuated vaccine for Indonesian QX-like IBVs (GI-19) using heat-adapted attenuation platform

Hyuk-chae Lee
KHAV

Infectious bronchitis virus (IBV) causes an acute and highly contagious respiratory, renal, and genital disease in chickens. Among structure proteins of IBV, S1 subunit of spike glycoprotein plays a major role in genotype and serotype determination. GI -15 and GI-19 IBV genotypes prevailed in South Korea and GI-15 IBV infection showed only respiratory signs in chicken, however, GI-19 IBV infection also showed nephron- and reproductive- pathogenic signs.We recently isolated IBV/Korea/289/2020 from chickens suspected IBV infection. We found it was recombined between GI-15 and GI-19, and breakpoint of the recombination was identified in middle of the S1 gene. As a result of pathogenicity and phenotypic characterization of it, the phenotype was more similar to the GI-19 genotype, not the GI-15. In cross-neutralization test for antigenic relatedness investigation, the recombinant virus also showed little or no difference with the GI-19 (77%) but major difference with the GI-15 (20%) in R-value. it was identified that N-terminal domain of the S1 gene is crucial to tissue tropism and target of neutralizing antibody, considering that the region including receptor binding domain of the recombinant was highly similar to the GI-19.In this study, we investigated pathogenicity and antigenicity of the recombinant IBV and it could be applied to development of next-generation vaccines such as vector vaccines in the future. However, we also identified the possibility of recombination between different IBV genotypes in field situations and it should be noted that accumulation of such recombination could lead to vaccine-break.

Development of a Highly-Sensitive NGS Method for Analysis of the Complete S1 Region of the Spike Gene from Infectious Bronchitis Viruses Directly from Field Samples

Derek Moormeier
Ceva Animal Health

Infectious bronchitis virus (IBV) is a highly-contagious coronavirus that causes significant upper respiratory disease, as well as nephritis and reproductive issues in poultry. The propensity of the viral genome to mutate and recombine results in the creation of novel variants that could escape immune responses generated by current live-attenuated vaccines. To determine the most appropriate preventative and protective measures for poultry flocks, it is routine practice in the poultry industry to submit samples from infectious bronchitis (IB) suspect cases for IBV detection, isolation, and/or genetic characterization using multiple different molecular methods. Given that the S1 region of the spike protein contains neutralizing and serotype-specific epitopes, we sought to develop an accurate and cost-effective method for sequencing the S1 region of the spike gene directly from IBV-positive field samples. By combining novel molecular protocols and custom bioinformatic tools, we assembled and analyzed complete IBV S1 genes/proteins directly from field samples, which helps avoid the potential lab bias that can be associated with chicken embryo propagation of IBV. This information can be used to type IBVs that are present in field samples, even those with low viral RNA levels and more than one virus. Analysis of this sequence data can help stakeholders to make informed decisions as it pertains to protecting their poultry flocks from IB.

Detection of Novel Infectious Bronchitis Viruses in Layers in Mexico and their Control by Using a Synergistic Live Vaccine Combination

Francisco Rios-Cambre
MSD Salud Animal Mexico

Infectious Bronchitis is caused by a gammacoronavirus which is highly contagious and is distributed worldwide causing serious economic burden in broilers and layers. The high capability of this RNA virus to mutate and recombine has caused the emergence of many serotypes, as well as the generation of antigenic variants that make their control very difficult by vaccination. The use of homologous vaccines for controlling these variants may also induce the emergence of novel variants. The concept of Protectotype, briefly, the use of two antigenically different vaccine viruses for controlling variants, has proven successful in many areas of the world, including the Mexican broiler industry. By using Sanger sequencing, we were able to detect at least five different novel IB strains that are not genetically related to any known IB virus strain. By applying the Protectotype combination of two strains a Massachusetts strain that spontaneously agglutinates chicken red blood cells, and another belonging to the 793-B serotype, we were able to demonstrate that all five novel variant strains were not detected anymore in the selected flocks where such viruses were initially found, and in all cases we were able to prove an improvement in the main production parameters in all vaccinated flocks.

Infectious Bursal Disease

Very virulent IBDV can efficiently replicate and be transmitted in layer-type naive chickens up to 16 weeks of age

Christophe Cazaban
Ceva Animal Health

Pathogenicity of infectious bursal disease viruses (IBDV) is less investigated for older layers, although some field cases are reported up to 12-15 weeks of age. The aim of our study was to investigate the changes in pathogenicity, shedding and transmission in an experimental setting to get more insights in age dependence and the possible role of older chickens in the circulation of IBDV strains in the field. Day-old commercial layers (Hy-Line Brown) were purchased without any vaccination in the hatchery. Different groups of 20 pullets were challenged at 4, 6, 8, 12 and 16 weeks of age. Each date, 5 non-challenged contacts were co-mingled with them to follow the horizontal transmission. A very virulent (vv) IBDV strain (D407/02/04 TR) was selected for the challenges in a dose of 5.0 log10EID50/chicken per os. All birds were followed for the presence of clinical signs and mortality for 10 days post-challenge (dpch). Cloacal swabs were collected at 3 and 10 dpch, bursa samples and serum samples at termination (10 dpch), both from the directly infected (seeder) and contact subgroups. IBDV amount in swab supernatants and organ homogenate was measured using a one-step real-time RT-PCR assay specific to vvIBDV (Peters et al., 2005). Bursa samples were submitted to histological investigation as well, serum samples were tested for IBDV antibody level by a commercial ELISA. Despite the decreasing mortality and morbidity rate with age, strong IBDV replication and efficient transmission to contacts was seen up to 16 weeks of age.

Comparison of IBD Antibody ELISA Titers using Kits from Various Vendors

Brenda Glidewell
Georgia Poultry Laboratory

Comparison of IBD Antibody ELISA Titers using Kits from Various VendorsApproximately 3,000 serum samples were taken from broilers and broiler breeders (22-26-wks of age and 55-62 wks) to compare IBD ELISA titers using kits from multiple vendors. Sera was collected from multiple flocks using different vaccination programs. Statistical analysis will be applied to

evaluate the differences in the data from the vaccination programs.

Developing a Novel Method for Sequencing and Genotyping of Infectious Bursal Disease Virus Directly from Field Samples

Julia McElreath
Ceva Animal Health

Infectious bursal disease virus (IBDV) is a ubiquitous virus that causes immunosuppression and mortality in poultry. It is common practice in the poultry industry to submit samples (primarily bursal tissues) that originated in the field for viral isolation, detection, and genomic classification of IBDV using classical and PCR laboratory methods. However, these methods can consume valuable time and resources. Thus, we sought to develop an accurate and cost-effective method for sequencing the hypervariable region within the VP2 gene of IBDV positive field samples, since this region encodes for the immunogenic capsid protein of the virion and is used for genotyping. By combining novel lab protocols, nanopore-based sequencing, and custom bioinformatic tools, we can sequence, assemble, and analyze the hypervariable region of the VP2 gene directly from field samples, even those with relatively low viral RNA levels. This information can be used to genotype IBDV that is present in field samples and culture. Analysis of this information can help stakeholders to make informed decisions as it pertains to protecting poultry flocks from IBD.

Presence and Distribution of Infectious Bursal Disease viruses belonging to Genotypes 2 and 5 in Mexico

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Infectious Bursal Disease virus (IBDV) is an abirnavirus that has affinity for the bursa of Fabricius, causing an immunosuppressive condition, Infectious Bursal Disease (IBD). IBDV is known to mutate and induce the emergence of antigenically divergent variant strains, which make control by vaccination difficult. Recently an effort was conducted to update the nomenclature of IBD viruses, by placing different isolates from several parts of the world into seven genogroups. Of these, viruses belonging to Genogroups 1, 2 and 5 were found in Mexico. Remarkably, Genogroup 5 has been detected only in Mexico, and it seems to be derived from a recombination of genetic material from viruses belonging to Genogroup 1 (dubbed “classic”) and Genogroup 2 (so-called Delaware E-type variant strains).For this paper we took samples from broiler flocks from Central, Western, and Southeastern Mexico. Imprints of bursal tissue from broilers and layer pullets at 21, 28, 35 and 42 days of age placed in FTA Cards were taken. The samples were then analyzed by PCR and those positive were subject to Next Generation Sequencing (NGS), so each positive sample could be placed in its corresponding Genogroup. The objective was to infer the geographical distribution of field viruses and determine in which regions viruses from Genogroup 5 were circulating at the sampling period. Bursal samples were also taken for histopathological analysis to correlate the level of bursal damage to which virus was detected.

Mycoplasma

Ms-H Vaccine (Vaxsafe MS) Protection in Broiler Breeders.

Francisco Perozo
Boehringer Ingelheim

Ms-H Vaccine (Vaxsafe MS) Protection in Broiler Breeders.Diego Murcia1; Gustavo Granados1; Diana Baquero2; Oscar Robin2 & Francisco Perozo 3 1Pollo Andino, Bogota Colombia.2Carval, Bogota Colombia.3 Boehringer Ingelheim, Bogota. Colombia..Mycoplasma sinoviae (MS) is

increasingly predominant in breeders and the etiologic agent responsible for aerosaculitis, synovitis and shell abnormalities, affecting the production and quality of the hatching egg. Eradication is the best way to keep the birds free from the disease; nevertheless, it is not economically plausible in South America. As an alternative vaccination (single dose of Ms-H) or antibiotic treatments are used trying to reduce or prevent clinical disease. The protection of the Ms-H vaccine was evaluated in broiler breeder flocks, assessing clinical disease, egg production, hatchability, antibody titer and presence of the wild type strain. The results obtained were compared with unvaccinated breeders' flocks treated with antibiotics reared in the same farm. Before vaccination rapid agglutination tests, ELISA and tracheal swabs PCR were performed to demonstrate MS negativity. Samples were taken at 13- and 30-weeks post vaccination to demonstrate the presence of the vaccine strain. A standard DIVA PCR test was used, which, given a positive result, makes it possible to differentiate whether the isolate corresponds to a wild type strain or a vaccine temperature-sensitive phenotype strain. The results obtained show significant differences between vaccinated and unvaccinated birds in terms of infection, hatchability and progeny behavior in the field suggesting the suitability of MsH vaccination for optimal MS control.

Monitoring of Mycoplasma gallisepticum live vaccine -K Strain –

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Mycoplasma gallisepticum (MG) is an important pathogen associated with poultry chronic respiratory disease leads to a considerable industry economic loss.Although biosecurity is ideal for MG control, but in large poultry populations, small geographic areas and multiple-age farms control by biosecurity alone is difficult and vaccine application is important. Middle East area is known with high challenge Mg due to multi-age and many farms at small areas, so it was worthwhile to monitor the efficacy of the K-strain live vaccine as previous studies showed its efficacy in poultry protection.2 Broiler Breeder flocks of 15000 birds/ each were vaccinated @ 4 weeks old:1st flock-spray and 2nd- eye drop, according to the vaccine leaflet instructions (a spray particle size adjusted approximately to 50 µm, eye drop dose is 0.03 ml/bird) serum samples were taken for "ELISA-BioChek/HI-in house-Vaxxinova-Japan/SPA-Charles River", trachea tissue for qPCR- BioChek, sequence analysis done via specialized institute. Samples were taken @ day of vaccination before vaccine administration, checking Mg free, @ 04, 06, 08, 10, 14, 30, 40, 50 and 60 weeks after vaccination.SPA results showed positive results starting from week 8 after vaccination, these results were compatible with ELISA, HI and PCR results. Titer values lower 1521, and 1010 while higher 7612 and 9261 for spray and eye drop respectively at 22 weeks after vaccination), compared to HI one lower 3 while higher 7 and 8 for spray and eye drop respectively. Ct values were 27/40, while eye drop 32/40 to 31/40). All results confirm compatibility with each other.Results of sequence and typing, of high Ct, confirmed the compatibility with the K5831B-19 vaccine strain.Live vaccine-K strain was able to multiply locally & gives a humOralimmunity at least until 26 weeks after vaccination, keep in mind that monitoring will continue until 60 weeks after vaccination. Key words: MG, Live vaccine-K-strainH. Bakri*; E. HallaqMonitoring of Mycoplasma gallisepticum live vaccine -K Strain –Vaxxinova, Amman 11118, Jordan *Presenter: husam.bakri@vaxxinova.com

Serological monitoring of chicken flocks Vaccinated against Mycoplasma gallisepticum

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Mycoplasma gallisepticum (MG) causes chronic respiratory disease in chickens. Especially, this disease results in severe economic losses due to reduced feed efficiency and drop in egg production in broiler breeders and layers. Therefore, live vaccines (ts-11, 6/85, F strain) have commonly been used to prevent the disease in the chicken flocks. In the present study, we conducted serological monitoring of the chicken flocks vaccinated with the live vaccines. A total of 2,298 blood sample were collected from chickens rearing in 11 flocks. The serological test for detection antibodies against MG was performed using commercially available ELISA kit (IDEXX, USA).MG infection was identified in 1(9.1%) non-vaccinated and 4(36.4%) vaccinated flocks. In the vaccinated flocks, the mean antibody titer of the antigen-negative flocks was 915±734 at 16~35 weeks of age. Titers of some ts-11 vaccinated flocks ranged from 1,100 to 2,800 at 20~30 weeks of age and were antibody negative thereafter.The flocks vaccinated with 6/85 and F-strains showed the level of below 400 until 56 weeks of age. Whereas, the mean antibody titers of antigen positive flock among vaccinate flocks were 386±543 (1 ~ 15 weeks of age), 1,404±886 (16~35 weeks of age),393±821 (36~55 weeks of age), and 7,981±5,102 (over 56 weeks of age). When the antigen was detected, the antibody titer was maintained above 1500 until 56 weeks of age.Consequently, the routine serological monitoring provides a due to the presence of MG field infection even in vaccinated chicken flocks.

Why not within first week? Success cases of early vaccination with a live Mycoplasma gallisepticum vaccine in Asia

Ludio Martins Gomes
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Why not within first week? Success cases of early vaccination with a live Mycoplasma gallisepticum vaccine in Asia. GOMES Ludio1, ZUANAZE Marcelo2 1,2 Vaxxinova – International The control of Mycoplasma gallisepticum (MG) should been primarily based on biosecurity measures and maintaining breeding stock free of infection, but in most Asian countries , due to the high MG prevalence within multiple-age farms, MG vaccines are massively used – mainly in long-lived chickens . Live vaccines can prevent production losses by allowing controlled exposure of flocks to low-virulence MG strains resulting in the development of immunity to subsequent field challenges. From an immunological perspective commonsense the vaccination should occur before the field challenge , meanwhile mostly of commercially available live vaccines , are recommended to be applied from 3 weeks of age onwards – sometimes too late for curbing the early MG challenge. The field study was conducted in 28 commercial layer farms in Nepal and Myanmar , to assess molecular epidemiology, clinical signs, and macroscopic lesions, of early vaccination (5d) with a live MG strain K5831 B-19 (K-Strain) in comparison with previous flocks vaccinated with F-strain. The results are still to be finalized within 2022 but from preliminary data, it was observed that at 6&23-weeks post vaccination, flocks vaccinated with K-strain showed 78 and 73% more vaccine strain persistence in upper respiratory sites respectively, 62% less clinical sings, in comparison with F-strain , and 15 times less at risk(OR)of having MG outbreak when using K-strain vaccine within the first week of age.

Newcastle Disease Virus

Newcastle disease virus genotypes circulating in Africa and their determinants of virulence: a systematic review

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Background: Since the discovery of Newcastle disease virus (NDV) in 1926, the emergence of new virulent genotypes from global epizootics and the year-to-year changes observed in the genomic sequence of NDV of low and high virulence implies that distinct genotypes of NDV are simultaneously evolving at different geographic locations across the globe. This vast genomic diversity may be favored by the large variety of avian species susceptible to NDV infection and by the availability of highly mobile wild bird reservoirs. The genomic diversity of NDV increases the possibility of diagnostic failures, resulting in unidentified infections. In this review, we will analyse the available literature on Newcastle disease virus genotypes in all 54 African countries, including the island nations of Mauritius, Seychelles, Comoros Island including Mayotte and Anjouan, as well as Cape Verde, Sao Tome and Principe, and La Reunion islands.Objective: The objective of this work is to identify the NDV genotypes circulating in Africa and map them; establish the evolutionary characteristics of the different genotypes of NDV circulating in Africa; and determine the virulence factors of the different genotypes of NDV in order to determine the evolution of the virus on the continent and its invasion dynamics. In this review, we will analyse the available literature in all 54 African countries, and La Reunion islands.Methods: In order to identify the different genotypes of the NDV already found in Africa, we will carry out a systematic review using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, based on both published and grey literature. Articles published between 1990 and 2021 will be used to obtain information on the different genotypes and virulence determinants of the NDVs and their geographical distribution in Africa. The geographical distribution of all the circulating genotypes in Africa will be mapped.

Evaluation of protection by ND vaccination protocols against early challenge with Velogenic Newcastle virus-VII.1 in commercial broiler with Maternal Immunity

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The aim of this work was to evaluate effectiveness of three different vaccination regime with inactivated NDV vaccine (MEFLUVAC-H5ND-7) (G-1 double inactivated ND vaccine at 1/10 day of age, G-2 single inactivated ND vaccine at 7-day of age and G-3 single inactivated ND at one-day of age) in-combination with three-dose from live ND vaccine against challenge with velogenic NDV (vNDV). 75,000 one-day old chicks obtained from commercial hatchery placed in three commercial broiler station “25,000 birds/station” and 40 one-day old chicks moved to Biosafety level-3 isolators (BSL-3) at MEVAC facility to serve as control groups (non-vaccinated group challenged “G-4” and non-vaccinated non-challenge-G-5). On weekly basis blood samples, cloacal swabs and oropharyngeal swabs collected check develop of Humeral immune response and exposure to any life-threatening Respiratory virus (Avian influenza” AIV”, NDV and infectious Bronchitis “IB” virus). At 26-day of age 15 birds from each station (G1-3) moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs collected and three birds from each group euthanized, and internal organs examined for three repeated times with 12 hours interval to ensure that birds free from any live threating viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 challenged with vNDV VII.1 (106 EID50) in 0.1 ml/ bird PSB via intranasal route and birds in G-5 received 0.1 ml PBS via intranasal route. Birds in G1-5 kept in BSL-3 for 10 days under observation and oropharyngeal swabs collected on 3, 6, 9 days post challenge (dpc). Regarding protection virus against mortalities following challenge with vNDV-VII.1; were 100% (15/15), 100% (15/15), 93.4% (14/15), 0% (0/15) and 100% (15/15) in

groups 1-5 respectively. Regarding virus shedding birds in G-1 showed significant lower shedding virus (amount of virus shedding and number of shedding in comparison to G2/3. In conclusion, using NDV inactivated vaccine in two-dose regime with live vaccines can provide protection to commercial broiler chicken against early challenge with vNDV in endemic areas as early as 28 days of age with significant lower shedding rate and the application of such regime can be effective tool in control NDV under vaccination strategy.

Epidemiological Surveillance of Avian Paramyxovirus-1 in Wild Birds from Perú

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Wild birds are the main reservoirs for the Newcastle disease virus, representing a risk to the poultry industry and biodiversity. This study evaluated the presence of the newcastle disease virus in wild birds, between September 2020 and November 2021. A total of 1,445 fecal samples of Charadriform birds were collected in northern Peru. The isolation of the virus was carried out in embryonated eggs of SPF chickens. The hemagglutinating activity of the allantoic fluid of the egg embryo was analyzed, and the presence of APMV-1 was confirmed by the IHA test. Six strains of avian Paramyxovirus 1 were isolated in two species of charadriform birds: Arenaria interpres (5 samples) and Pluvialis squatorola (1 sample). These strains will be evaluated by molecular methods to determine their pathogenicity and their relationship with other strains previously isolated in Peru.

The Thermal Stability of Newcastle Disease Virus in Poultry Litter

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Disposal of contaminated organic material during recovery from an animal disease outbreak is costly and laborious and must minimize the risk of disease spread. Understanding the thermal stability of avian paramyxovirus type-1 (APMV-1), the causative agent of Newcastle disease in poultry, will help inform risk assessments. In warm environments heat may be utilized to help decontaminate a premises. Therefore, the objective of this study was to characterize the thermal stability of APMV-1 in poultry litter. Virus inactivation was evaluated at 10.0°C through 43.3°C, at 5.5°C intervals (50-110°F in 10°F intervals) using the I2 isolate of APMV-1 which is a vaccine strain that is known to be highly stable. A high titer of virus was added to used chicken litter containing wood shavings ("poultry litter"). Litter with both low and high moisture levels were evaluated. Samples were collected from artificially contaminated litter samples at different time intervals then were titrated in embryonated chicken eggs to quantify infectious virus. At high temperatures infectious virus could not be detected within a couple of days to a week, whereas at lower temperatures, it took up to 112 days. Furthermore, virus in high moisture litter generally inactivated faster compared to low moisture litter. The decimal reduction time (D-value) was also shorter in high moisture litter. Overall, the thermal inactivation profile for NDV from this study provided a guideline for the time required to inactivate NDV at specific temperature conditions in organic material.

Parasitology

Toxoplasma gondii Prevalence in Waterfowl and Gulls From Eight States in the United States

Richard Gerhold
University of Tennessee College of Veterinary Medicine

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite Toxoplasma gondii, which infects mammals and birds. This study tested a total of 391 serum samples from 15 wild bird species collected from 2009 to 2019 from eight states for T. gondii antibodies using the modified agglutination test and found a seroprevalence of 26.6% (104/391). In addition, PCR was performed on heart tissues from 72 gull and waterfowl samples from Pennsylvania and Tennessee, and a prevalence of 13.9% (10/72) was documented. Tennessee (27/39, 69.2%) and Pennsylvania (18/23, 78.3%) had higher T. gondii seroprevalence compared to the rest of the states while Texas (12/83, 14.5%) and Minnesota (25/154, 16.2%) had lower seroprevalence. Hooded mergansers (9/9, 100%) and Ring billed gull (18/23, 78.3%) had significantly higher T. gondii seroprevalence while Mallards (38/202, 18.8%) and Blue winged teal (14/90, 15.6%) had lower T. gondii seroprevalence than the rest of the species (P<0.001). Sex of the bird did not have any significant effect on the exposure to T. gondii (P=0.714) while adults (52/205, 25.4%) had higher seroprevalence (P=0.012) compared to juveniles (17/127, 13.4%). Positive waterfowl were found in areas with higher precipitation (4.5670 ±2.47644 in.) and negative waterfowl were found in low precipitation areas (3.7745±2.54413 in.). Confounding data had some limitation on our statistical analysis is addressed in the discussion of this paper. Further research is needed to elucidate the transmission dynamics of T. gondii in waterfowl, gulls and other aquatic animals and to determine any potential zoonotic risk.

Metabolic profile of Histomonas meleagridis and undefined bacterial population in Dwyer's media with and without rice starch

Richard Gerhold
University of Tennessee

Histomonas meleagridis is considered one of the most important pathogens for domestic and wild turkeys as well as replacement pullets. As previously reported, a starch source and serum are essential components to propagate H. meleagridis in vitro in Dwyer's media. To investigate the role of rice starch in Dwyer's media, we conducted a metabolic analysis of intracellular metabolites of H. meleagridis, and bacteria grown in Dwyer's media with (SD) and without rice starch (NR). Metabolites were measured using ultra performance liquid chromatography-high resolution mass spectrometry. Dwyer's media with white rice starch significantly supported the growth of H. meleagridis in comparison to NR media at 42, 66, 114, 142, 166 hours while bacterial growth was not affected by the type of the media used at these time points. From the SD and NR media, a total of 170 known metabolites were detected. Almost all the metabolites decreased in NR media compared to SD media at 66-142 hours. There was a significant difference in metabolites at 0 hours. Upon was further investigation, and we found that riboflavin recorded the highest variable importance in projection score (VIP) that was significant at all five components at blank and 0 hours. In the future, we will be investigating the role of riboflavin and if media supplemented with riboflavin can support H. meleagridis growth in absence of rice starch. Furthermore, these findings have potential for discovering chemotherapeutic agents to control and prevent blackhead.

Salmonella

Effect of AviPro™ Megan™ Vac 1 on growth performance in broiler chicks Oraly challenged with S. Thyphimurium wild strain

Priscilla Karina Koerich
PRISCILLA KOERICH

Salmonella enterica serovar Typhimurium is one of most prevalent serovars in poultry operations worldwide, identified as one of the most

important foodborne pathogens. Infected broiler chicken can become a key source for carcass contamination at slaughter that threatens public health and reputation of the poultry industry. Vaccination of birds against Salmonella is the main pilar in a strategy to prevent these infections and reduce the risks to public health. Live attenuated Salmonella Typhimurium (ST) vaccines can confer protection against salmonellosis by inducing both cell-mediated and mucosal immune response. In this study, several Salmonella enterica serovar Typhimurium challenge models were tested to identify the best conditions under which to perform the experimental infection of 2-weeks-old broilers and evaluate the performance impacts in birds vaccinated with live-ST vaccine (AviPro™ Megan™ Vac 1). The treatments in the study were the following: T1, non-vaccinated, non-challenged; T2, ST-vaccinated, non-challenged; T3, ST-challenged; T4, ST-challenged. The trial was performed with 3600 birds, allocated within six groups with 12 replicates, containing 50 birds per unit. ST challenge was inoculated at the 14 days of age (D14) by Oralgavage with dose of 1.0 mL (109 CFU). The vaccine improved weight gain in 60 grams when compared with the treatment T3, ST-challenged and non-vaccinated at 28 days (1.672 x 1.612 grams), ($P<0.05$). There was no influence in performance parameters in those birds receiving ST-live vaccine and no challenged. Beyond the protective effect associated to broiler immunization against major foodborne pathogens such as Salmonella Typhimurium, the adoption of AviPro™ Megan™ Vac 1 also confers economic advantages by improving weight gain.

Efficacy evaluation of Salmonella Enteritidis SRP vaccine against challenge by Salmonella Enteritidis, S. Typhimurium and S. Gallinarum in brown laying hens

Gabrielle Bragaglia
Vaxxinova

Salmonella serotypes continue to be important concerns in poultry. A novel vaccine technology has been developed based on purified siderophore receptors and porin proteins (SRP) - Vaxxon-SRP SE, by Vaxxinova. Vaccine efficacy was evaluated against challenge by Salmonella Enteritidis (SE), S. Typhimurium (STM) and S. Gallinarum (SG) in commercial brown layer-hens. Each vaccinated challenged chicken group had the respective correlated control group. Hens were vaccinated with 2 doses of Vaxxon-SRP SE at 9 and 14 weeks old and challenged at 18 weeks old. ELISA results demonstrated a high serological titer response for vaccinated groups before challenge. For hens challenged with SE, bacterial cecal count had a significant reduction of 1.6, 1.6, 1.0 and 1.4 log10 at 4-, 7-, 11- and 14-days post-inoculation (DPI) in vaccinated group. For the groups challenged with STM, a significant reduction of 1.1 and 1.7 log10 in vaccinated group was observed at moments 7 and 11 DPI, respectively. For the fecal excretion parameter there was also a significant difference between the vaccinated and unvaccinated groups, with a reduction of 48% and 53% of positivity, respectively for birds challenged with SE and STM, in comparison with unvaccinated group. For groups challenged with SG there was a significant reduction in mortality rates, reaching 10% in group vaccinated by subcutaneous route and 3% in group vaccinated by intramuscular route, significantly different from 47% noticed in unvaccinated group. Thus, survival rate reached satisfactory level of protection, considered equal to or greater than 90% for vaccinated hens. Vaccination with Vaxxon-SRP SE provided lower fecal shedding and systemic infection in hens infected with SE and STM and reduced mortality in hens infected with SG, suggesting capacity to control salmonellosis in the fields.

Epidemiological investigation of S. typhimurium monophasic at a broiler integrator

Louise Dufour-Zavala
GPLZN

Epidemiological investigation of S. typhimurium monophasic at a broiler integratorKat Muro, Doug Waltman, Louise Dufour-Zavala, Dave FernandezOver time, the Salmonella serotypes found at a broiler integrator changed from a variety on environmental Salmonella species to almost exclusively 1,4,(5),12:i:-The presence of S. typhimurium monophasic (1,12:i:-) in pullets and breeders was investigated. Pullet production parameters and interventions were investigated as risk factors associated with the detection of different Salmonella serotypes, but with 1,12:i:-in particular. The persistence of 1,12:i:- throughout the production system to the processing plant was also analyzed, and results will be presented.

Quantitative Distribution and Interaction of Salmonella Zega with Host Cells in Visceral Organs of Chickens Infected through Three Routes

Fakilahyel Mshelbwala
Federal University of Agriculture, Abeokuta

Immunohistochemical studies of the visceral organs of chickens experimentally infected with Salmonella Zega by three routes was carried out to compare the quantitative distribution and interaction of the organism with host cells. The Salmonella Zega was isolated from a natural outbreak of salmonellosis and serotyped at an Italian Reference Laboratory. 100 day-old chicks were raised to two weeks; the birds were then divided into 4 groups of 25 each. Group A was inoculated Oraly, group B was inoculated intraperitoneally, group C were administered per cloaca and group D were not inoculated and served as control. All the infected birds were inoculated with 0.2 ml of 1 x 108 cfu of the bacteria. Two birds from each group were sacrificed every 24 hours post infection. Sample of visceral organs were collected for immunohistochemical studies. The distribution of Salmonella Zega in every organ was taken as mean ± SD of the number of foci of immunoreactions and compared using a 2-way ANOVA. The interaction of Salmonella Zega was determined by taking the percentage of the days' post infection in which immunoreactions were detected in host cells in each route of infection. The distribution of the organism was highest in the lung of intraperitoneally infected chickens (83.95 ±27.89) and lowest in the heart (5.21 ±3.65) of chickens that were infected per cloaca. The highest percentage of interaction of Salmonella Zega was recorded in the epithelial (100%) and blood (100%) cells in all the routes of infection. There were variations in the distribution of Salmonella Zega in visceral organs of chickens but the level of interactions with host cells were similar even when infected through different routes.

Safety of two Salmonella vaccines and zootechnical performance after vaccination of laying hens for prevention of salmonellosis

Jeniffer PImenta
Vaxxinova

Safety of two Salmonella vaccines and zootechnical performance after vaccination of laying hens for prevention of salmonellosisJeniffer G. F. PimentaA, Gustavo Schaefer A, Hítalo J. S. Barbosa B, Leonardo J. C. Lara B.A Vaxxinova®, Vargem Grande Paulista - SP, 06730-000, Brazil. B Federal University of Minas Gerais, Department of Animal Science, Belo Horizonte-MG 31270-901, Brazil. Salmonellosis is among the diseases of long-cycle commercial birds that cause economic losses and highlight risks related to public health. According to the scientific literature, there are more than 2500 serovars described, and Salmonella Gallinarum and Salmonella

Enteritidis serovars come up with relevance, being responsible for typhoid and paratyphoid infections in poultry flocks. Poultry birds' vaccination is a form of prevention and a premise of biosecurity that occurs earlier in the rearing phase and helps the management avoid challenges in the production phase. Our objective was the safety evaluation of the vaccination within 35 (thirty-five) days of bird's release in free cages for protection against salmonellosis and the monitoring of the zootechnical performance during the entire rearing phase. It had three experimental groups a first controlled one without vaccination the second experiment within the inactivated vaccine protection against Salmonella Enteritidis and Salmonella Gallinarum so as another protection group against Salmonella Gallinarum associated with viral antigens vaccines were provided twice for birds 35 to 105 days old the safety of the monitored vaccines besides the zootechnical performance body weight, batch uniformity, feed intake, feed conversion then were verified viability rate. All 1350 birds evaluated on vaccinated groups were not affected on performance indicators. There was no clinical occurrence of the disease. The welfare of birds and biosecurity was improving with lesser management during the evaluated phase.

Isolation of Salmonella Spp from Trachea of Broiler Chickens and Analysis of its Potential Role as Monitoring Diagnostic Tool

Martha Pulido Landinez
Mississippi State University

Previous experiences recovering Salmonella spp from chicken's trachea evaluated the hypothesis that transmission by the fecal–respiratory route may be a viable portal of entry for Salmonella in poultry. In previous unpublished studies performed at the MSU PRDL, it was possible to isolate Salmonella spp from tracheal samples of broiler pullets, broiler breeders, and broiler chickens. Considering tracheal swabs could be a good not-invasive sample to recover Salmonella spp from commercial birds, a study using a convenience sampling method was performed collecting tracheal swabs from 100 PRDL broiler chicken necropsies, during five months. Tracheal swabs were collected before necropsy from five chickens in each case and inoculated on Tetrathionate enrichment broth. Five tracheal swabs per case were also streaked on plates (Blood and MacConkey agars). Salmonella spp isolation followed NPIP isolation procedure. Colonies suggesting Salmonella were further identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a VITEK® MS instrument. All Salmonella spp isolates were genotyped by Intergenic Sequence Ribotyping (ISR). If Salmonella spp is isolated from other organs in the same case, this isolate will be also genotyped and a comparison with the one isolated from trachea will be performed. Analysis of the usefulness of this sampling method and the results of the study will be discussed during the presentation.

Detection of Salmonella in Quail Eggs for Ambulatory sale in Districts of the City of Lima-Peru

Magali Salas
ALFA BIOL S.A.C

Detection of Salmonella in quail eggs for ambulatory sale in districts of the city of Lima-PerúMagali Salas M.1, Ana Claudia Zamora.2, Nilda Castro A.3, Nicole Lazo.4, Aldo Cedrón A.5, Iván Camargo C.6, Carla Bardález C.7The aim of this study was to estimate the prevalence of Salmonella spp. in quail egg samples destined to human consumption on 17 districts of Metropolitan Lima. 1,200 eggs were analyzed from 40 different seller points of quail eggs, a product that is habitual consume of kids in scholar age and is frequently seen being sold in ambulatory posts. The eggs were analyzed in a pool of 30 eggs per sample, through the ISO

6579-1: 2017 method for Salmonella spp. detection for both superficial surface and internal content. There was found one strain from El Agustino, which is the fifth district with most child and adolescent population in Lima. The strain was isolated from superficial surface and identified as belonging of the C2 group of Salmonella spp and is currently in process of genotyping. This study is looking to support the national health entities with useful information.

Changes in Salmonella Serotypes over the last 30-something years

Doug Waltman
GPLZN

Vaccinology

Duration of Ultifend® & Rispens protection against Newcastle disease virus

Olivia Faulkner
Ceva

A vectored vaccine containing turkey herpesvirus (rHVT) with dual insert of infectious bursal disease (IBD) and Newcastle disease (ND) antigens, referred to here as Ultifend®, was developed in combination with Marek's disease virus, Serotype 1 (Rispens). For this study, protection of the ND antigen in the vectored vaccine fraction of Ultifend® & Rispens was evaluated against Newcastle disease virus (NDV) Texas GB challenge. The objective of this study was to evaluate whether subcutaneous (SQ) administration of Ultifend® & Rispens in layer chickens positive for maternal antibodies against NDV was effective against preventing disease caused by the NDV challenge. Chickens (n=114) were challenged with NDV at 30 or 60 weeks of age. Seroconversion against IBD and ND were monitored using an ELISA at 0, 2, 4, 6, 8, 12, 16, 20, 30, and 60 weeks after vaccination. The chickens were observed following challenge for mortality. Sample to positive (S/P) ratio of IBD and ND antibody titers were similar in control chickens and Ultifend® & Rispens vaccinated chickens at 4 weeks after vaccination indicating maternal antibody presence. Antibody titers of Ultifend® & Rispens vaccinated chickens increased above the control chickens by 6 weeks after vaccination and remained elevated for the remainder of the study. In the unvaccinated control group, the challenge caused mortality in 90% of the chickens at 30 weeks of age and 97% of the chickens at 60 weeks of age. The SQ administration of Ultifend® & Rispens to chickens with maternal antibodies to NDV prevented mortality in 93% of the vaccinated birds at 30 weeks after vaccination and in 96% of the vaccinated birds after 60 weeks.

Comparison of the Serologic Response of Two Vaccination Programs with Different IBV Antigen Fractions in Broiler Breeders

Antonio Cobian
SPM, NCSU

Comparison of the Serologic Response of Two Vaccination Programs with Different IBV Antigen Fractions in Broiler BreedersCobian, Antonio11Production Breeder Manager, RedondosIn broiler breeder production, vaccination against the main poultry diseases is key for success not only for the current flock being vaccinated but also, it's progeny. Vaccination programs in breeders, specially in Peru, are created to achieve the hiperimmunisation of the birds. Vaccines are carefully evaluated considering their composition and immunogenicity, before fully including them to the program. With new products being launched, some with different and interesting antigenic fractions, is our responsibility to run efficient field trials that can lead to effective decision making. To achieve this, data analysis is key to obtain objective conclusions. In this study, the objective was to compare the serologic response of two vaccination programs with different IBV

antigenic fractions, in broiler breeders. For the statistical data analysis and data visualization, the Python programming language coupled with Numpy/Scipy modules was used. This is an example of the use of the Real World Evidence methodology applied in the poultry industry.

Evaluating IBV and NDV Monovalent or Bi-valent Vaccinations Take by On-Site PCR Test

Keat Fu
Aviagen Inc.

Infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) are important respiratory diseases in the poultry farm. The vaccines for NDV and IBV are either mono-valent (separate) or Bi-valent (combined) administrated via spraying method at the hatchery for day old chicks. This study will demonstrate the interference potential of IBV to NDV by using on-site, fully automatic PCR test in the farm. Group A day old chicks were sprayed with IBV plus NDV live vaccine (bi-valent), and group B were sprayed with mono-valent IBV, NDV live vaccine separately. After vaccination, we collected ten trachea swab samples from the two groups at day 7. The samples were tested individually with the POCKIT TM Central IBV reagent set and NDV LaSota strain reagent set. We calculated the PCR positive rate of IBV and NDV, and the high positive rate means good vaccination uniformity (=80%). In group A, the positive rates of IBV and NDV were = 80%. In group B, the positive rates of IBV were 100%, whereas NDV were lower than 50%. In this study, it was demonstrated that IBV vaccine virus affects the NDV vaccine virus replication in group B. It was due to equal amount of virus in mono-valent vaccine, IBV will replicate faster and interfere NDV. However, for bi-valent vaccine of IB and ND with well formulated more ND virus quantity to offset IBV interference resulting the uniformity of vaccine take were good.

Development of an indirect Enzyme-Linked Immunosorbent Assay based on ILT gD recombinant protein for the monitoring of HVT-gD vaccine.

Marina Gaimard
Innovative Diagnostics

Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by the infectious laryngotracheitis virus called Gallid herpesvirus 1. ILT leads to major losses because of mortality and/or decreased egg production. Vaccination is an essential tool for poultry disease control. Different types of vaccines are commercially available. Conventional vaccines (TCO and CEO), based on native virus, offer good protection but can produce latent infections and reactivation of the virus in the field. Vector vaccines are created by genetic modification(s) of vector microorganisms and the integration into their genomes of exogenous gene(s) encoding for immunogenic protein(s) from viruses responsible of diseases of interest. In the case of poultry vector vaccines, the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT) are commonly used as vector virus. Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, Innovative Diagnostics has developed an indirect ELISA for the monitoring of rHVT-ILT gD vaccine. A sequence of the ILT glycoprotein gD was synthetized and expressed in a baculovirus system to produce specific antigen. Purification steps was carried out, and the final purified gD protein used to develop an indirect ELISA. The performances of this indirect ELISA were evaluated with experimental and field samples vaccinated with rHVT-ILT gD vaccine. The following poster summarizes the preliminary validation data obtained for this gD indirect ELISA.

Compatibility of A Recombinant HVT-IBD Vaccine with CVI to Provide Protection Against

Very Virulent Marek's and Very Virulent IBDV Challenge in SPF Birds

Angela Hartman
Zoetis

Due to the increasing presence of circulating very virulent (vv) and vv+ Marek's isolates in the field, the combination of HVT vectored vaccines such as HVT-IBD with a Rispens vaccine is critical to provide protection especially in long lived layers and breeders. This study assessed the combination of HVT-IBD with a CVI988 Rispens vaccine either in ovo or subcutaneous at hatch on the protection provided against a Day 5 vvMDV RB1B challenge and a Day 14 vvIBD challenge in SPFs. Compatibility was demonstrated for vvIBD and showed 75% efficacy following subcutaneous administration and 80% efficacy following in ovo injection. Compared to previous studies utilizing the HVT-IBD vaccine alone, that have shown 90-93% efficacy against vvIBD, there may have been a small degree of temporary interference, which may have related to the Day 14 challenge and early competition of HVT-IBD and Rispens vaccines to colonize the target cells, T lymphocytes. However, the clinical protection against a very virulent IBD challenge was still very strong. The combined vaccines showed 96% and 98% protection against vvMDV following in ovo and subcutaneous at hatch administration, respectively. Thus, the combination of HVT-IBD with a CVI-988 vaccine provides a strong compatible solution for protecting against circulating field challenges.

Construction of DIVA capable, low cytotoxic-endotoxic, and immune-competent attenuated Salmonella Gallinarium vaccine candidate

John Hwa Lee
Jeonbuk National University

This study describes the generation and characterization of a novel low cytotoxic and endotoxic Salmonella Gallinarium live attenuated vaccine for the prevention of fowl typhoid in chicken. The strain consists of three deletions of virulence genes namely lon, cpxR, and rfaL that bring the strain attenuation, efficient antigen presentation without leaving a disease in the host. The strain attributes DIVA capability due to O-antigen deficiency generated by rfaL deletion. On top of these mutations, SG pagL locus has been replaced by lpxE gene originated from Francisella tularensis to detoxify intact lipid A. Characterization of detoxified SG strain (SGVSdt; SG ?lon ?cpxR ?rfaL?pagL::lpxE) revealed, the strain is well tolerated by baby chicken at 7 days old Oraly at 1 x 108 CFU/birds and subcutaneously at the age of 14 days at 1 x 107 CFU/bird. Parenteral immunization was completely free from environmental contamination and did not contaminate eggs at sexual maturity. Immunization via subcutaneous (SC) route derived comparable levels of humOraland cell-mediated immune responses marked by Th1 skewed responses similar to the commercial vaccine strain SG9R and the protection efficacy was well comparable to SG9R. Compared to SG9R and the parent strain SGVS, the detoxified SGVSdt generated a significantly low level of inflammatory responses marked by cytokines such as IFN-? (high) and TNF-a (Low) that was also evident by histopathological analysis. Upon virulent challenge, 100 % survival was acquired for both SG9R and SGVSdt, and the level of protection was well evident in H & E stained histopathological assessment marked by reduced tissue damages, lesions, and inflammation signs. Overall this detoxified Salmonella Gallinarium strain can be a far improved candidate superior to the current commercial strain SG9R due to no effect on growth and productivity of chicken yet with improved safety and comparable level of protection efficacy.

New technology vaccines return on investment in commercial broilers under high challenge field condition

Hazem Negm
Ceva Animal Health

New technology vaccines return on investment in commercial broilers under high challenge field condition.Hazem Negm1, Francois Roulleau2, Christophe Cazaban2, Alaa Fattouh11Ceva Animal Health, Riyadh, Saudi Arabia, 2Ceva Animal Health, Libourne, France.Moving forward toward hatchery vaccination has been made easier and more widely spread by the new technology (Vector as well as immune-complex) vaccines. Hatchery vaccination enables using less farm vaccination; in addition, it ensures a more controlled vaccination process and vaccination audit system. A field trial involving 10 million broilers has been conducted throughout one full year comparing birds vaccinated with IBD immune-complex vaccine in combination with rHVT-ND vector vaccine s/c at the hatchery vs Live intermediate IBD vaccine at the hatchery and farm with ND inactivated vaccine at the hatchery. The added value of the new vaccination program has been measured by the differences on several production parameters (Mortality rate difference, Body weight differences and feed conversion rate difference). Comparing the two-vaccination program under the same conditions for 7 consecutive production cycles provided an average of improved mortality rate by 2.3% and 44 grams more of body weight with 0.025 better feed conversion rate leading to extra revenue 0.19 $ per bird for the hatchery vaccination with the new technology vaccines. As a result of less vaccination rate in the farm by replacing 2 intermediate IBD vaccines with Immunocomplex IBD vaccine in combination with the rHVT-ND vector vaccine induced a better control of both diseases as reflected on the overall performances of the flocks. New technology hatchery vaccines also help in reinforcing biosecurity measures by decreasing the traffic of vaccination crews to the farms.

Determining Minimum Protective Dose of Inactivated Trivalent Fowl Adenovirus (4, 8b, 11) Vaccine

Dam-Hee Park
Choong Ang Vaccine Laboratories

Fowl Adenovirus (FAdV) infection has been reported from many different countries causing high mortality in young chicks emphasizing the importance of vaccination. FAdV is comprised of 12 serotypes (1~8a, 8b, 9~11). The types reported as prevalent in South Korea are FAdV-4, FAdV-8b, and FAdV-11. In this study, inactivated trivalent fowl adenovirus (4, 11) vaccine was manufactured to determine minimum protective dose. 6-week-old SPF chickens were vaccinated with dose of 10^7.5, 7.0 TCID50/dose. Blood samples were collected after 3-week-post vaccination to execute serum neutralization test. After blood collection, chickens were challenged via intravenous route. According to the data, high neutralizing antibody titer and full protection were observed starting from 10^7.0 TCID50/dose. The result indicates vaccination of inactivated trivalent FAdV vaccine would be supportive for prevention of FAdV in Korea.

Virology

Molecular characterization of circulating avian reovirus in Egypt

Ayman El-Deeb
Evapharma

Avian reovirus is one of the most important viruses causing economic problems in poultry industry worldwide beside its vertical transmission, which increases its importance in breeder flocks. In the present study, the circulating strains of avian reovirus in both

broiler and broiler breeder flocks in Egypt were detected using real-time RT-PCR in many governorates representing the different epidemiological sectors in Egypt. The positive samples undergo conventional RT-PCR followed by sequencing and phylogenetic analysis based on partial nucleotide sequences of the S gene, which revealed that the obtained ARV isolates grouped into many different groups, however, most of the detected isolates were belonging to the S1113-like cluster of ARV and displayed 100%identity with Chinese MSO1 isolate and 98.7% with S1133 vaccinal strain. The obtained results indicated continuous circulation of ARV in poultry flocks in Egypt under vaccination pressure, which may increase the possibility of the virus mutation by both point mutation as well as genetic reassortment. Whole genomic sequences of the circulating viruses should be done to study all mutations and its impact on virus virulence.

Pathological investigation on Fowl Adeno virus infection in Middle East Area 2019-2021

Husam Al Bakri
Vaxxinova International BV

Fowl adenoviruses (FAdV), members of the genus Aviadenovirus, are important infectious pathogens associated with inclusion body hepatitis, hydropericardium hepatitis syndrome (HHS) and gizzard erosion in chickens and other birds, leading to substantial economic losses for the poultry industry worldwide. Recently, in the Middle East it was noticed high mortality associated with FAdV clinical signs. Therefore, it was notable to perform a survey on the presence of FAdV in the area during a period 2019-October 2021.173 samples were taken from liver/ Trachea/ Feather/Spleen from different countries in the Middle East (Jordan/Syria/ Lebanon/ Iraq/UAE/ Qatar/Kuwait/SA) for qPCR analysis (Kylt and Kogene). 89% of samples showed positive results, 34% of the latter positive results showed low Ct values. Therefore, they were sent to a specialized institute for phylogenetic typing. 32% of samples with low Ct were able to give phylogenetic typing positive results and the following serotypes were able to be identified (59% FAdV-E, serotype8b,/ 35% FAdV-D,serotype11/ 6% FAdV-C-serotype4). Different serotypes of FAdV were playing a role in the region.

Fowl Adenovirus Strain Identification Challenge

Christophe Cazaban
Ceva Animal Health

The concurrent presence of different Fowl adenovirus (FAdV) species (named from FAdV A to E respectively) in a case or pathological sample is not an extraordinary phenomenon, but it might have been remained frequently undetected using the conventional methodologies. Diagnosing of FAdV-caused diseases routinely involves the identification of the infecting virus strain, mainly using a conventional PCR, which targets a portion of pol or hex gene (Kajan et al., 2011; Hess, 2010), thus providing the possibility of broad range detection and identification upon Sanger-sequencing of the obtained PCR product. It can be performed directly on the organ samples or – following propagation on cell culture – on virus isolates. This approach, however, may reveal only the abundant virus type. Nonetheless, during the isolation process, the ratio of the concurrent species/serotypes may change, with a potential profound effect on the characteristics of the isolate. Both the immunological and pathological properties can be affected by an unnoticed co-existence in the sample or the altering ratio of different viruses in the isolate. To sensitively and timely detect the potentially present different species/serotypes in the organ samples, even if one of them is in minority, we utilize two approaches: i) ‘deep sequencing’: NGS-based multiple sequencing of the pol gene amplicon and ii) the set of species-specific qPCR

assays, independently detecting the most frequent species, i.e., FAdV A, C, D, E and additionally in the latter case serotypes FAdV-8a and 8b, respectively. The agreement of the results of deep sequencing and the set of qPCR assays was confirmed by parallel testing of organ samples as well as consecutive passages of virus isolates. We believe that the presented approaches are inevitable for providing accurate diagnosis, as well as for proper strain identification and characterization for autogenous vaccine production.

Retrospective Study of Tumors of Unknown Origin in Broiler Breeders in the USA (2011-2020)

Baxter Elliot
North Carolina State University College of Veterinary Medicine

Retrospective study of tumors of unknown origin in broiler breeders in the USA (2011-2020)Baxter A. Elliot, Allison C. Boone, Aneg L. Cortes, Tahseen Aziz, Isabel M. GimenoSporadic lymphoid tumors of unknown etiology have been described in chickens. In some cases, they have been linked to the presence of fully replicating endogenous retroviruses. In others, the etiology remains unknown. In this study, we conducted a retrospective study (January 2011 until February 2020) of all the cases of commercial chickens that were submitted to the North Carolina Veterinary Diagnostic Laboratory System (NCVDLS) and were diagnosed as lymphoid leucosis (LL) and/or other sarcomas. Furthermore, we have included six additional cases in which further molecular diagnosis to rule out Marek's disease, reticuloendotheliosis, and the most common exogenous Avian Leucosis viruses was conducted at NCSU. All cases reported in this study were from broiler breeders. There were 42 cases that were diagnosed as LL (28), LL and sarcomas (4), and other type of sarcomas, mainly fibrosarcomas and histiocytic sarcomas (10) by the NCVDLS. From 2011 until 2016, the incidence was low (1-2 cases per year) but it increased in 2017 (6 cases), 2018 (19 cases), and 2019 (10 cases). The information from 2020 covers only two months and there was one case. All cases submitted to NCSU reported high frequency of tumors in the mortality of flocks with no health nor production problems. Histopathology revealed either LL alone or LL and histiocytic sarcomas. Oncogenic MDV genome was at latency levels and no proviral DNA or ALV-A, ALV-B/D, ALV-J, and REV was found. The role of endogenous retroviruses and how SB-1 vaccine can increase the incidence of such tumors will be discussed.

Diverse Single-stranded DNA Viruses Identified in Chicken Buccal Swabs

Darrell Kapczynski
USDA-ARS-Southeast Poultry Research Laboratory, Athens, Ga

High-throughput sequencing approaches offer the possibility to better understand the complex microbial communities associated with humans and animals. Viral metagenomics has facilitated the discovery and identification of many known and unknown viruses that inhabit mucosal surfaces of the body and extend our knowledge related to virus diversity. In this study, we applied metagenomics sequencing to chicken buccal swab samples and identified novel unclassified small DNA viruses with circular genome organization. Out of 134 viral-like circular putative genome sequences identified, 70 are cressdnaviruses and 26 are microviruses, whilst the remaining 38 most probably represent sub-genomic molecules. Cressdnaviruses found in this study belong to the Circoviridae, Genomoviridae and Smacoviridae families as well as previously described CRESS1 and naryavirus groups. Among these, genomoviruses and smacoviruses were the most prevalent across the samples. Interestingly, we also identified 26 bacteriophages that belong to Microviridae family whose members are known

to infect enterobacteria. These studies increase our general knowledge of viruses that infect or are associated with the upper respiratory track of commercial chickens. Further investigation is warranted to determine the prevalence of these viruses and their pathology, if any, in birds.

Understanding the Respiratory virome of backyard poultry in Minnesota

Anita Kumari
University of Minnesota

Since the Minnesota outbreaks of Highly Pathogenic Avian Influenza (HPAI) in 2015, the Minnesota Veterinary Diagnostic Laboratory (MVDL) has seen an increase in the number of phone calls, emails, and cases received from small flock owners (SFOs) regarding respiratory disease in their flocks. Many SFOs are concerned that any respiratory sign is due to HPAI, when in most cases infectious larynogotracheitis virus (ILTV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and/or Mycoplasma gallisepticum (MG) have been identified alone or as a mixed infection. SFOs need to know about common pathogens that may affect their birds and how to control them. Tracheal/choanal swabs were collected from chicken flocks (3-4 birds in each flock) in Minnesota counties that were historically either positive or negative for HPAI in 2015 and pools of these swab samples were analyzed for respiratory virome by Next-Generation Sequencing (NGS). The data of 15 samples have been analyzed and based on this analysis, thee infectious laryngotracheitis virus was detected in most of the respiratory cases. Different viruses such as chicken picornaviruses, gemycircularvirus, avian leukosis virus, endogenous retroviruses, and bacteriophages were detected in clinically healthy birds. The analysis of remaining 25 samples is in process and data will be presented in the conference. This information will be useful in improving small flock health and will help to identify potential risks to both small flocks and commercial poultry.

Pathogenicity Evaluation of a Turkey Coronavirus Isolate (TCoV NC1743) in Turkey Poults for Establishing a TCoV Disease Model

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Turkey coronavirus (TCoV) can cause a highly contagious enteric disease in turkeys with severe economic losses in the global turkey industry. To date, no commercial vaccines are available for control of the disease. In the present study, we isolated a field strain (NC1743) of TCoV and evaluated its pathogenicity in specific-pathogen-free (SPF) turkey poults to establish a TCoV disease model. The results showed that the TCoV NC1743 isolate was pathogenic to turkey poults with a minimal infectious dose at 106 EID50/bird. About 50% of one-day-old SPF turkeys infected with the virus's minimal infectious dose exhibited typical enteric disease signs and lesions from 6 days post-infection (dpi) to the end of the experiment (21 dpi). In contrast, fewer than 20% of older turkeys (1- or 2-week-old) infected with the same amount of TCoV displayed enteric disease signs, which disappeared after 15-18 dpi. Although all infected turkeys, regardless of age, shed TCoV, the older turkeys shed less virus than the younger birds, and 50% of the 2-week-old birds even cleared the virus at 21 dpi. Furthermore, the viral infection caused day-old turkeys more body-weight-gain reduction than older birds. The overall data demonstrated that the TCoV NC1743 isolate is a highly pathogenic strain and younger turkeys are more susceptible to TCoV infection than older birds. Thus, one-day-old turkeys infected with the minimal infectious dose of TCoV NC1743 could be used as a TCoV disease model to study the disease pathogenesis, and the TCoV NC1743 strain could be used as a challenge virus to evaluate a vaccine protective efficacy.

Wealth of Knowledge

Reduction of Chondronecrosis with Osteomyelitis Lameness in Broilers Fed Metal Amino Acid Complexes Using Two Challenge Models

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Zinpro

The benefits of supplementing trace minerals to animals go far beyond the improvements seen in animal performance and production. Trace minerals such as zinc (Zn), manganese (Mn) and copper (Cu) participate in several physiological processes of the body, and thereby can also directly impact animal health and welfare. In this presentation, we will explain how a commercial combination of organic trace minerals Zn, Mn and Cu (Availa-ZMC), helped broilers to control BCO lameness, a bacterial infection that causes necrosis of rapidly growing bones like tibia and femora. The objective of the study was to evaluate the efficacy of an in-feed supplementation of Availa-ZMC in reducing BCO lameness in broilers under two BCO-challenge models. In the first model, chicks were placed in suspended wire flooring to induce BCO lameness through mechanical stress. In the pathogen exposure model, pens contained wood shaving litter and only the chicks belonging to the source pens were challenged with Staphylococcus agnetis str. 908 in the drinking water. Both models were evaluated for 56 days. The dietary treatments consisted of a control group receiving only inorganic sources of zinc, manganese, and copper; a second group in which the inorganic sources were partially replaced with normal levels of Availa-ZMC; and a third group receiving a replacement with higher levels of Availa-ZMC. The two dietary treatments containing Availa-ZMC were effective in reducing lameness by 20% in the wire flooring model and 25% in the litter flooring model. The reduction of lameness was attributed to the improved intestinal barrier integrity and efficacious immune response mediated by increased expression of tight junction proteins, immunomodulatory cytokines and stimulation of bactericidal killing of adherent peripheral blood monocytes obtained from the birds treated with Availa-ZMC. In conclusion, Availa-ZMC has the potential to provide reasonably effective alternative to antibiotics to mitigate BCO lameness in broilers.

Microbiota of the poultry litter beetle (Alphitobius diaperinus)

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The litter beetle (lesser mealworm) is the predominant insect pest within poultry houses. Beetles cause structural damage to the houses, and the consumption of too many beetles by the birds can lead to indigestion and impaired feed conversion. Most importantly, the litter beetle can carry a wide variety of pathogens. Litter beetles are frequently resistant to pesticides. Therefore research into alternative methods of population control are necessary. Knowledge of the beetles' bacterial microbiota might offer insight for investigating control of beetle populations by identifying bacteria that are pathogenic for the beetles or by targeting important symbionts.The objective of this investigation was to characterize bacteria associated with beetles. First, bacterial counts in beetle homogenates and rinses were determined by Uni-Bacterial qPCR. The copy numbers in rinses and homogenates were not significantly different. Secondly, the microbiota of beetles cultured in the lab and beetles caught at the Auburn University Poultry Research Farm were determined by 16S-rRNA sequencing. Bacteria of five different classes were associated with cultured beetles; the most abundant were Bacilli, Gammaproteobacteria and Mollicutes. The wild-caught beetles harbored bacteria of 19 different classes and thus a significantly higher

diversity. Taken together, results indicate beetles do not harbor a large number of bacteria inside their bodies and thus consequently, the bacteria that are associated with them are highly dependent on their environment. These results need to be confirmed by other methods, but it seems unlikely that manipulating the bacterial microbiota to control litter beetles is a promising approach.

Why Did the Student Cross the Road: Influencing Factors to Become a Poultry Veterinarian

Linda Flores
Western University of Health Sciences

Few, if any, veterinary schools teach poultry medicine in the veterinary curriculum. Often, poultry medicine is an elective course for students. Hence, the objective of this study was to investigate at what point in time do students get exposed to poultry medicine and what factors influence their decision to pursue a career as a poultry health professional. To achieve this objective, a survey was developed and distributed to poultry health professionals in order to determine potential factors that would influence a career choice. Results indicated that once believed popular expectations do not necessarily lead to a career in poultry. For example, growing up with poultry exposure does not impact an individual's decision to decide to be involved in a future career with poultry. This finding along with other factors will be further discussed. Our findings demonstrated that a student's background does not hinder one to pursue their dream job and provides hope that students could become poultry professionals if given the optimum opportunity.

Women Pioneers in the Poultry Field 1900-Present

Jessica Hockaday
Hockaday Consulting

The AAAP Women's Network (AWN) Committee will be featuring women pioneers in the poultry industry and present their accomplishments in the form of a poster. The focus of the poster will be on women that are/were outstanding researchers, veterinarians, and leaders in their prospective areas of the industry. These women will be chosen based on their contributions to the advancements in science-based knowledge, expertise, and education on poultry health, welfare, and food safety and how they have shaped the poultry industry as a whole. The goal of the poster is to highlight scientific and leadership contributions made by women members of AAAP, World Poultry Veterinary Association (WPVA) and the World's Poultry Science Association (WPSA) and how they have advanced the global poultry industry. The poster will depict the last 100+ years of history, spotlighting professional biographies of women pioneers, some names you may know and others you may be hearing of for the first time, and how their professional efforts shaped and continue to advance an ever-changing industry. The information gathered from this poster will be incorporated into the AAAP Avian Medicine Committee's biography database as well, which will enable the program to continue to follow its' mission, "to collect, permanently preserve, organize, and publicize information concerning the history of the AAAP and poultry health in order to advance the profession through an appreciation of its heritage." We look forward to sharing their stories and contributions to the poultry industry, and provide this information to AAAP membership at large.

Biosecurity and supporting measures to improve its implementation in poultry farms

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Biosecurity measures represent a powerful tool for the prevention of infectious diseases, avoiding or limiting the introduction and spread

of pathogens in poultry farms. Despite their well-known relevance, their proper implementation is not always achieved. Within the EU NetPoulSafe project (G.A. No. 101000728), an in-depth investigation and analysis of biosecurity compliance in European poultry production is being carried out. Three different questionnaires have been designed to collect data on biosecurity and supporting measures for biosecurity implementation in three different stakeholders' categories in seven EU Countries participating to the project. In Italy, advisors (n=37), farmers (n=30), and operators (n=9) were interviewed during summer 2021. In detail, farmers and advisors of conventional broiler (n=5) and layer (n=6), free range broiler (n=3) and layer (n=3), turkey (n=7), duck (n=3), and breeder (n=3) farms were interviewed, as well as operators working in slaughterhouses (n=2), hatcheries (n=3), egg collection facilities (n=2) and feed suppliers (n=2). Data were analyzed by using descriptive statistics to assess the overall biosecurity compliance and to identify the supporting measures required to improve biosecurity levels. Preliminary findings show a good level of biosecurity compliance in Italian poultry farms. Responses to the questionnaires were comparable between advisors and farmers among the different productive categories; however, education and training of stakeholders seem to be still necessary. Analyses are currently carried out to identify the most promising supporting measures to help improving biosecurity compliance.

Health challenges and interventions in small flock poultry and waterfowl

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Keeping small flock poultry has steadily increased in popularity. However, veterinary care remains complicated as many veterinarians have limited experience working with poultry, and poultry medicine has historically focused on commercial flocks. Small flocks have different health challenges often relating to lack of vaccination, increased pathogen exposure, and old age. Our goal was to better understand small flock health by determining common causes of death, medical interventions taken, and any changes in this over time. A retrospective case series will be performed using records from the Alabama State Diagnostic Laboratory System. Case records from the years 2009-2011 and 2019-2021 from the four Alabama laboratories will be included. Provided case histories and laboratory diagnoses will be analyzed and cataloged. In a pilot analysis of 2019 data, 103 submissions were reviewed. Ninety-four submissions were chickens, the remainder were peafowl, turkeys, ducks, and guineafowl. In chickens, the most common complaint was of non-specific general malaise. Medical intervention (including prohibited antibiotics) was provided in 27.7% of submissions. The three top diagnoses were Marek's disease, respiratory disease, and peritonitis. In the other species, high mortality and general malaise were common complaints, and 54.5% of submissions were affected by one or more types of internal parasites. Once the additional years of data are analyzed trends will be better recognized, especially in the less common species. It is expected that when comparing the time periods, recent years will have greater numbers of chickens submitted, with more evidence of veterinary intervention.

Biosecurity as a Promising Tool to Reduce Antimicrobial Use in Poultry Farms

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Antimicrobial use (AMU) in poultry farms represents a potential threat to public health that drives the emergence and spread of antimicrobial resistance. Alternatives to reduce AMU are warranted, and implementation of biosecurity measures is one of the best

alternatives to this purpose. The aim of this study was to correlate the AMU with the implementation of biosecurity measures in broiler farms. Fifty-two conventional broiler farms located in a densely populated poultry area in Northern Italy were investigated in 2020. In each farm, biosecurity compliance was assessed by mean of checklists, while AMU was calculated using the Defined Daily Dose Animal for Italy (DDDait). The correlation between biosecurity measures and AMU was determined using logistic regression analysis. Results show that compliance with biosecurity measures (e.g., presence of a gate at the entrance of the farm or presence of documents certifying cleaning and disinfection of vehicles coming from the slaughterhouse) seems to reduce AMU, meanwhile breaches in biosecurity (e.g., parking inside the farm or direct/indirect evidence of the presence of pests in the farm or loading of silos inside the farm) might increase the AMU. The findings of this study show that compliance with biosecurity is positively correlated with decreased AMU, suggesting that greater attention should be paid to the compliance of biosecurity measures in broiler farms.

Welfare

Understanding stress and welfare of laying hen pullets using stocking density and feeder space stressors

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The laying hen pullet phase is a crucial time period establishing parameters that will impact the laying hen throughout the production cycle. However, there are few concrete management guidelines and no formal welfare guidelines during the pullet phase especially in cage-free housing. This study used Lohmann Brown Lite and Lohmann LSL-Lite pullets housed on the floor at high, medium, and low stocking densities and provided with 3.5 or 7.1 cm of feeder space to identify indicators of stress and poor welfare. The goal of this research is to identify changes in the birds' physiology, immunology, outward appearance, and production for use as stress or welfare markers. These markers can then be used to guide management choices on farm and improve both production and welfare of the pullets.

The Influence of Mirrors as Environmental Enrichment on Pullet Behavior

Grace Sims
Auburn University

Concerns for animal welfare have led to increased scrutiny of the layer industry. Introducing environmental objects to stimulate the pullets in cage free environments can be argued to enrich and improve the bird’s life. The objective of the present experiment was to use mirrors and measure interaction to indicate whether this was a meaningful source of enrichment.Two identical floor pens were used, each containing 100 pullets. Cameras were set up in three different locations at each pen. Mirrors were introduced at 8 days of age and removed at 22 days of age. Starting at 29 days of age, mirrors alternated two days in and then two days out for two weeks. Seven days were chosen to observe the recording footage. Data was collected at each hour of the days chosen, and 19 different pullet behaviors were measured. Additionally, novel object fear tests were performed to measure the latency to pullet’s response when a foreign object was placed into the pen. This fear test was conducted 10 times for both pens at regular intervals. In preliminary analysis, there was little influence of the presence or absence of the mirrors on the behavior of the pullets. Few chicks were seen interacting with the mirror. However, a slight increase in the interaction of the mirror as the pullets aged was noted. Based on these observations, mirrors do not seem to be a

meaningful environmental enrichment. For the novel object tests, there is a noticeable trend that the pullets became less fearful the more times the test was conducted. The time taken for them to approach the object decreased significantly. There was no significant difference between pens or days with and without mirrors.