▶**AAAP Symposium**
Investigating Disease and Assessing Productivity Using Epidemiological Tools

AUGUST 2-5 | 2019

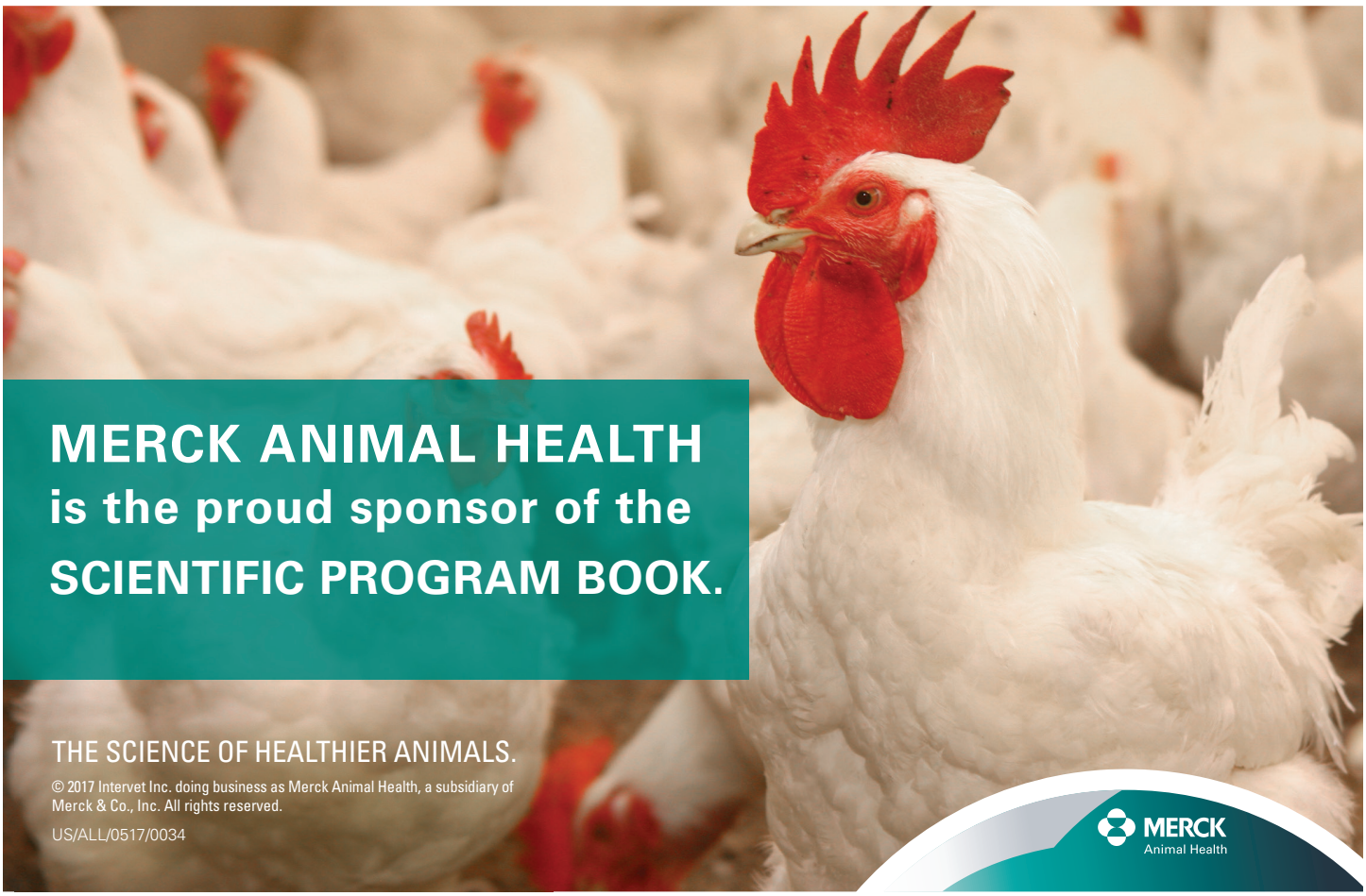
AAAP

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.

Washington, D.C.
August 2-5, 2019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AAAP Event Schedule

Friday

Opening Session
8:00 AM: Renaissance Ballroom West AB
Renaissance Hotel

Keynote Address
Oscar Fletcher
8:20 AM: Renaissance Ballroom West AB
Renaissance Hotel

Opening Welcome and Poster Reception
5:00 PM: Congressional Ballroom AB
Renaissance Hotel

Saturday

Scientific Symposium
8:00 AM: Renaissance Ballroom West AB
Renaissance Hotel

Committee Roundtables
12:45 PM: Mount Vernon Square B
Renaissance Hotel

Poultry Professional Networking And Meet the Expert
5:30 PM: Renaissance Foyer
Renaissance Hotel

Sunday

Yoga
6:30 AM: Meeting Room 3
Renaissance Hotel

Wellness Talk
Mike Gergye
10:15 AM: Renaissance Ballroom West AB
Renaissance Hotel

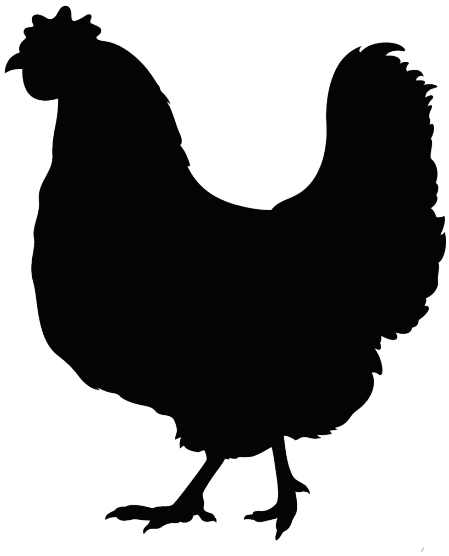
Business Meeting
10:45 AM: Renaissance Ballroom West AB
Renaissance Hotel

Women's Network Dinner
6:30 PM: Meeting Rooms 12-14
Renaissance Hotel

Monday

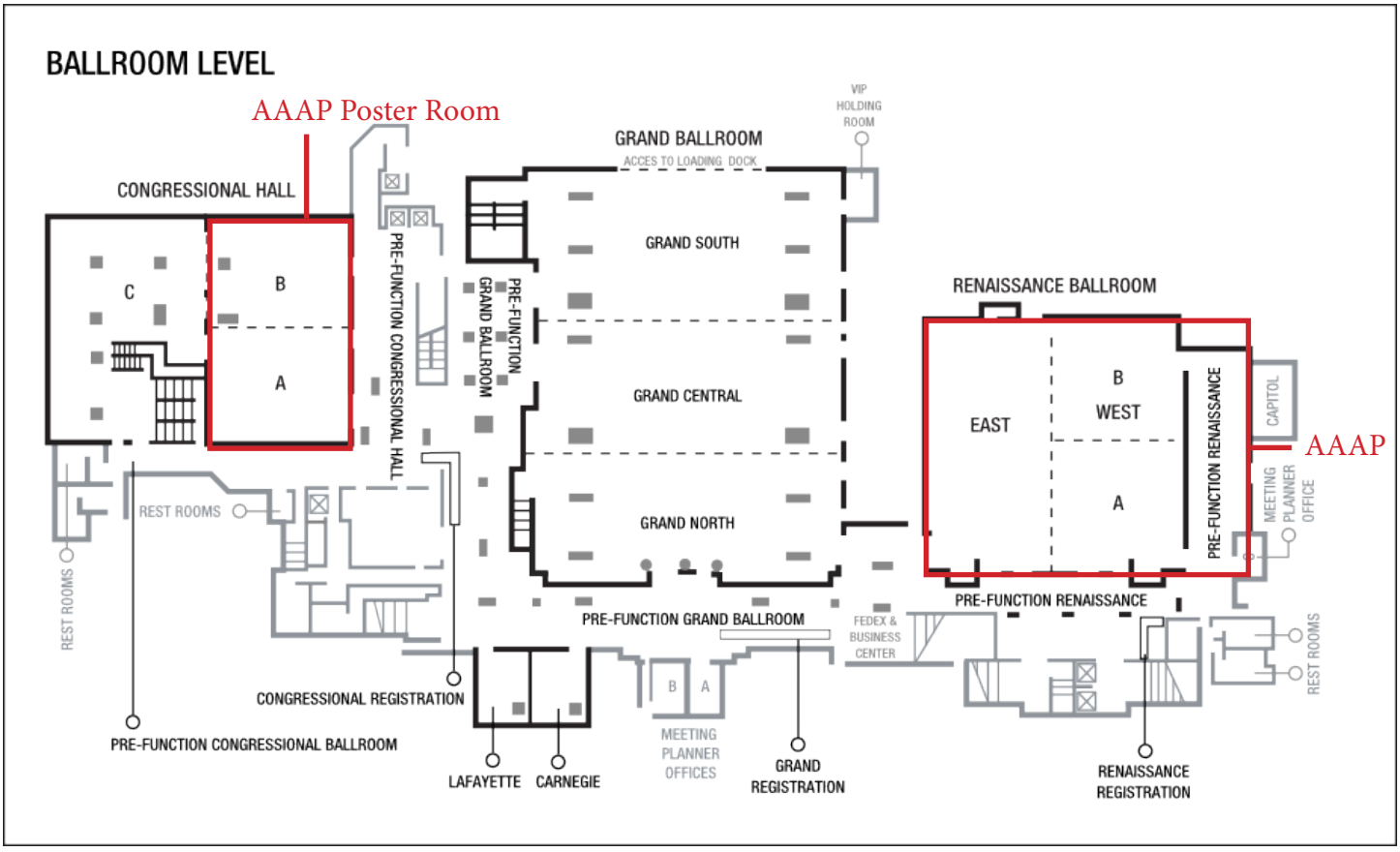
History Lecture
K.V. Nagaraja
11:30 AM: Renaissance Ballroom West AB
Renaissance Hotel

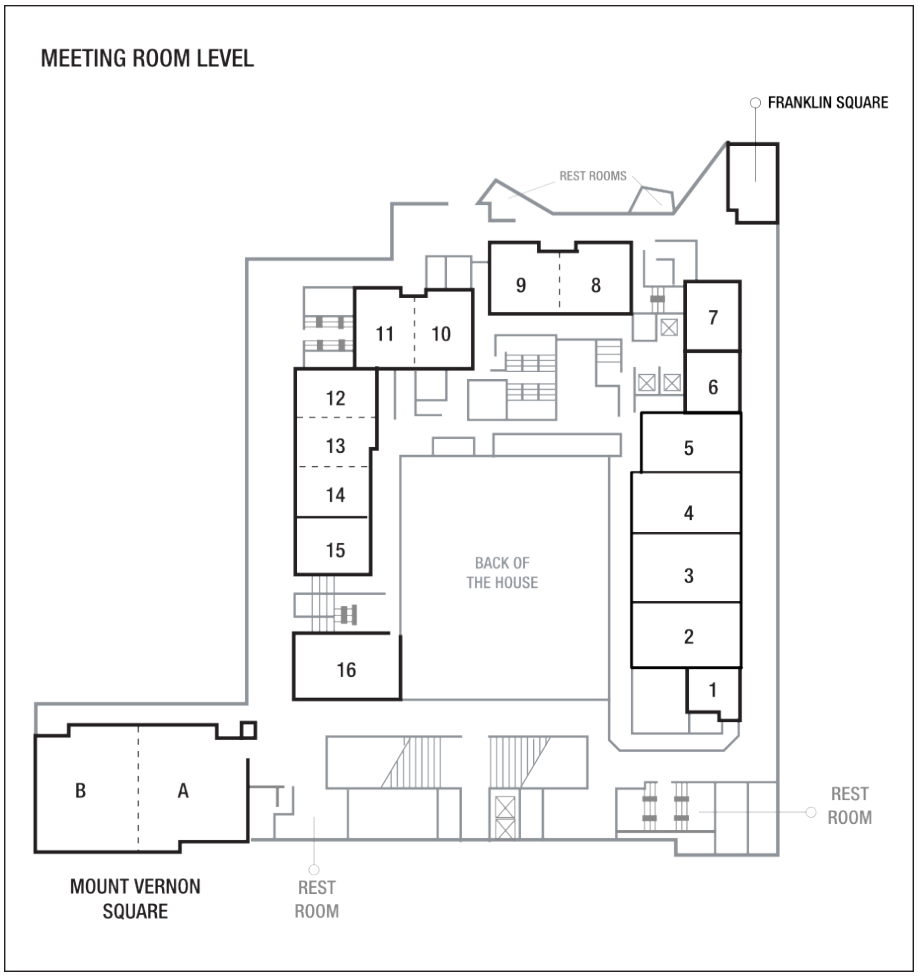
Awards Reception & Dinner
5:30 PM: Congressional Ballroom Prefunction
6:00 PM: Congressional Ballroom AB
Renaissance Hotel



Name of Group	Meeting Date	Beg. Time	End Time	Location	Room
AAAP Board of Directors Meetings					
AAAP Inc. and Foundation Board of Directors	Thursday, August 1	7:00 AM	5:00 PM	Renaissance Hotel	Meeting Room 6
AAAP Past Presidents Luncheon	Friday, August 2	12:00 PM	1:30 PM	Renaissance Hotel	Meeting Room 2
AAAP Committee Chairs and BOD Meeting	Monday, August 5	12:30 PM	5:15 PM	Renaissance Hotel	Meeting Room 5
Committee Meetings					
Histopathology/Case Report Interest Group	Thursday, August 1	1:00 PM	5:00 PM	Renaissance Hotel	Meeting Room 8/9
Small Flocks Interest Group	Thursday, August 1	1:00 PM	5:00 PM	Renaissance Hotel	Meeting Room 3
AAAP Auditing Committee	Friday, August 2	4:00 PM	5:00 PM	Renaissance Hotel	Meeting Room 4
Awards Committee	Saturday, August 3	1:30 PM	2:25 PM	Renaissance Hotel	Meeting Room 6
Avian Diseases Advisory Board	Saturday, August 3	1:30 PM	2:25 PM	Renaissance Hotel	Meeting Room 5
Avian Diseases Manual Editorial Board	Saturday, August 3	1:30 PM	2:25 PM	Renaissance Hotel	Meeting Room 7
Digital Communications Committee	Saturday, August 3	1:30 PM	2:25 PM	Renaissance Hotel	Meeting Room 8
Outreach Committee	Saturday, August 3	1:30 PM	2:25 PM	Renaissance Hotel	Meeting Room 9
Toxic, Infectious, Miscellaneous & Emerging Diseases Committee	Saturday, August 3	1:30 PM	3:25 PM	Renaissance Hotel	Congressional Ballroom C
Avian Diseases Editorial Board	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Meeting Room 5
Enteric Diseases Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Mount Vernon Square A
Epidemiology Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Renaissance Ballroom East
Membership Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Meeting Room 2
Preceptorship Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Meeting Room 10
Research Priorities Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Meeting Room 8
Small Flocks Committee	Saturday, August 3	2:30 PM	3:25 PM	Renaissance Hotel	Meeting Room 4
Diseases of Public Health Significance	Saturday, August 3	3:30 PM	4:25 PM	Renaissance Hotel	Congressional Ballroom C
Drugs and Therapeutics Committee	Saturday, August 3	3:30 PM	4:25 PM	Renaissance Hotel	Meeting Room 4
Education Committee	Saturday, August 3	3:30 PM	4:25 PM	Renaissance Hotel	Meeting Room 3
History Committee	Saturday, August 3	3:30 PM	4:25 PM	Renaissance Hotel	Meeting Room 10
LAC Committee	Saturday, August 3	3:30 PM	4:25 PM	Renaissance Hotel	Meeting Room 8
Animal Welfare Committee	Saturday, August 3	3:30 PM	5:25 PM	Renaissance Hotel	Mount Vernon Square A
Respiratory Diseases Committee	Saturday, August 3	3:30 PM	5:25 PM	Renaissance Hotel	Mount Vernon Square B
Food Safety Committee	Saturday, August 3	4:30 PM	5:25 PM	Renaissance Hotel	Meeting Room 6
Tumor Virus Committee	Saturday, August 3	4:30 PM	5:25 PM	Renaissance Hotel	Congressional Ballroom C
AAAP Foundation Scholarship Committee	Sunday, August 4	7:00 AM	8:00 AM	Renaissance Hotel	Meeting Room 10
AAAP Foundation Development Committee	Monday, August 5	9:00 AM	10:00 AM	Renaissance Hotel	Meeting Room 5
Program Events					
AAAP Opening Session	Friday, August 2	8:00 AM	8:30 AM	Renaissance Hotel	Renaissance Ballroom West AB
2019 Scientific Program Keynote Address	Friday, August 2	8:20 AM	9:00 AM	Renaissance Hotel	Renaissance Ballroom West AB
AAAP Welcome Reception and Poster Session	Friday, August 2	5:00 PM	6:30 PM	Renaissance Hotel	Congressional Ballroom AB
Mentor/Mentee Breakfast (Invitation Only)	Saturday, August 3	7:00 AM	8:00 AM	Renaissance Hotel	Penn Quarter
AAAP Symposium	Saturday, August 3	8:00 AM	12:00 PM	Renaissance Hotel	Renaissance Ballroom West AB
BOD/Committee Chair Meeting	Saturday, August 3	12:15 PM	12:45 PM	Renaissance Hotel	Meeting Room 3
Committee Roundtables	Saturday, August 3	12:45 PM	1:30 PM	Renaissance Hotel	Mount Vernon Square B
AAAP Poultry Professional Networking (Meet the Expert)	Saturday, August 3	5:30 PM	6:30 PM	Renaissance Hotel	Renaissance Ballroom Foyer
AAAP Women's Network Dinner and Meeting	Saturday, August 3	6:30 PM	8:30 PM	Renaissance Hotel	Meeting Rooms 12-14
Yoga/Walk	Sunday, August 4	6:30 AM	7:30 AM	Renaissance Hotel	Meeting Room 3
Wellness Talk	Sunday, August 4	10:15 AM	10:45 AM	Renaissance Hotel	Renaissance Ballroom West AB
AAAP Business Meeting	Sunday, August 4	10:45 AM	12:00 PM	Renaissance Hotel	Renaissance Ballroom West AB
AAAP Lasher-Eckroade History Lecture	Monday, August 5	11:30 AM	12:00 PM	Renaissance Hotel	Renaissance Ballroom West AB
AAAP Awards Reception before Dinner	Monday, August 5	5:30 PM	6:00 PM	Renaissance Hotel	Congressional Ballroom Prefunction
AAAP Awards Dinner	Monday, August 5	6:00 PM	8:00 PM	Renaissance Hotel	Congressional Ballroom AB
ACPV					
ACPV Exam Practice	Wednesday, July 31	5:00 PM	8:00 PM	Renaissance Hotel	Penn Quarter
ACPV Exam #1	Thursday, August 1	7:00 AM	5:30 PM	Renaissance Hotel	Penn Quarter
ACPV Exam #2	Thursday, August 1	7:00 AM	5:30 PM	Renaissance Hotel	Meeting Room 10/11
ACPV Exam Grading Room	Thursday, August 1	7:00 AM	10:00 PM	Renaissance Hotel	Meeting Room 7
ACPV Board of Governors Meeting	Sunday, August 4	7:00 AM	10:00 AM	Renaissance Hotel	Meeting Room 16
ACPV Reception/Annual Meeting	Monday, August 5	7:00 AM	8:30 AM	Renaissance Hotel	Congressional Ballroom C
Invitation Only					
Association of Veterinarians in Broiler Production Meeting	Thursday, August 1	8:00 AM	3:00 PM	Renaissance Hotel	Meeting Room 5
Association of Veterinarians in Egg Production	Thursday, August 1	12:00 PM	5:00 PM	Marriott Marquis	Liberty Ballroom L
Association of Poultry Consultants and Independent Laboratories	Thursday, August 1	1:00 PM	5:00 PM	Renaissance Hotel	Meeting Room 4
Association of Veterinarians in Turkey Production	Thursday, August 1	8:00 AM	5:00 PM	Marriott Marquis	Georgetown University
NC Veterinarians and NCSU Students and Alumni	Friday, August 2	7:00 AM	8:00 AM	Renaissance Hotel	Mount Vernon A
Association of Poultry Primary Breeder Veterinarians	Saturday, August 3	11:30 AM	1:30 PM	Renaissance Hotel	Meeting Room 16
MAM and MAHM Alumni Association	Sunday, August 4	5:00 PM	7:00 PM	Renaissance Hotel	Congressional Ballroom C

Renaissance Hotel





2018-2019 AAAP Foundation Board of Directors

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Director - Western 2020

Maritza Tamayo
Director-at-Large 2019

Jarra Jagne
Director-at-Large 2020

John Smith
Past President 2019

AAAP Representatives to AVMA

AVMA Animal Agriculture Liaison
Andrea Zedek 2021
Joel Cline 2021 (Alternate)

AVMA Animal Welfare Committee
Katherine Weathers 2019
Helen Wojinski 2019 (Alternate)

AVMA Clinical Practitioners Advisory Committee
Hector M. Cervantes 2020
Megan Lighty 2021 (Alternate)

AVMA Committee for Disaster and Emergency Issues
Brian Wooming 2019

AVMA Committee on Antimicrobials
Randall Singer 2021
Hector Cervantes 2021 (Alternate)

AVMAConvention Education Program Committee
Ivan R. Alvarado 2021
Natalie Armour 2021 (Alternate)

AVMA Food Safety Advisory Committee
Douglas Fulnecheck 2020
Daniel Wilson 2019 (Alternate)

AVMA House of Delegates
Gregg J. Cutler 2019
Katherine Weathers 2019 (Alternate)

AVMA Legislative Advisory Committee
Suzanne Y. Dougherty 2021
Jose Linares 2019 (Alternate)

AAAP Representatives to Allied Organizations

Council for Agriculture Science and Technology (CAST)
Robert D. Evans 2019
Mohamed El-Gazzar 2019

Professional Animal Auditor Certification Organization (PAACO)
David A. Pyle 2020
David R. Hermes 2019

Stakeholder Forum on Antimicrobial Resistance (S-FAR)
Randall Singer 2019

United States Animal Health Association (USAHA)
Eric Gingerich 2019

Transmissible Diseases of Poultry and Other Avian Species
Dale C. Laurer, Chair

Animal Agriculture Coalition (AAC)
Jose Linares 2020

Animal Agriculture Alliance (AAA)
Donald Ritter 2022

National Chicken Council (NCC)
Mark Burleson 2022

United Egg Producers (UEP)
Elizabeth Beilke 2022

US Poultry & Egg (USP)
Bruce Stewart-Brown 2022

AAAP Foundation Committee Chairs

Awards
Andrea Zedek 2019

Kenneth Eskelund Preceptorship
Francene S. Van Sambeek 2020

Poultry Scholarship
Mark Bland 2019

Development Committee
Kristen Roza-Sutherland 2020
Andrea Zedek 2021

Avian Bioscience Travel Scholarship
Erica Spackman 2023

AAAP Constitutional Committee Chairs

www.aaap.info/committees

Auditing	Nominating	Resolutions
Karen Burns Grogan	Francene Van Sambeek	Frederic J. Hoerr

AAAP Task Force Committee Chairs

Animal Welfare and Management Practices
Kenneth Opengart 2019

Diseases of Public Health Significance
Dustin Burch 2022

Drugs and Therapeutics
Timmothy Cummings 2022

Education
Monique Franca 2019

Enteric Diseases
John Schleifer 2019

Epidemiology
Dave Fernandez 2020

Food Safety
Brett Hopkins 2021

History of Avian Medicine
Patricia Dunn 2019

Legislative Advisory
Suzanne Y. Dougherty 2021

Membership
Geoffrey Lossie 2019

Program Advisory
Ivan Alvarado 2021

Respiratory Diseases
Alejandro Banda 2022

Research Priorities
Natalie Armour 2021

Tumor Virus
John Dunn 2019

Toxic, Infectious, Miscellaneous and Emerging Diseases
Milos Markis 2022

Small Flocks
Victoria Bowes 2019

Women's Network
Sara Steinlage 2019

Outreach
Bernard Beckman 2023

Committee Review
Bruce Stewart-Brown

2018-2019 AAAP Board of Directors

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Eric Jensen
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Suzanne Dougherty
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Director - South 2022

Bernard J. Beckman
Director - Central 2019

Rocio Crespo
Director - Western 2020

Jarra Jagne
Director-at-Large 2020

Maritza Tamayo
Director-at-Large 2019

John Smith
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Avian Diseases Editorial Board
Y. M. Saif

Avian Diseases Manual
Martine Boulianne

Avian Histopathology Manual Board
Tahseen Abdul-Aziz

Gross Pathology of Avian Disease
Tahseen Abdul-Aziz

Biosecurity Manual Board
Robert L. Owen

Diseases of Poultry Editorial Board
David E. Swayne

Isolation of Avian Pathogens Manual Board
Susan Williams

Friday, August 2, 2019

Room	Renaissance Ballroom West		
8:00 AM	**Opening Session**		
8:20 AM	**<u>Session Keynote</u>: Cases Selected from a Career in Poultry** Oscar Fletcher, *NC State University*		
Room	**Renaissance Ballroom East**		**Renaissance Ballroom West**
Topic	**Enteric Health**		**Case Report**
Moderator	Hyun Lillehoj		Enrique Montiel
9:00 AM	**Investigations of Influencing Factors on the Gut Health and Microflora Composition in Layers** Silke Rautenschlein, *Clinic for Poultry, University of Veterinary Medicine, Hannover*		**Respiratory Disease and Bacterial Septicemia of Broilers Linked to Three Specific Breeder Flocks** David French, *Sanderson Farms, Inc.*
9:15 AM	**The Effects of Direct-Fed Microbial Supplementation, as an Alternative to Antibiotics, on Growth Performance, Intestinal Immunity, and Epithelial Barrier Integrity in Broiler Chickens Infected with Eimeria maxima** Inkyung Park, *USDA-ARS*		**Respiratory Disease and Bacterial Septicemia of Broilers Linked to Three Specific Breeder Flocks** Jose Linares, *Ceva Animal Health*
9:30 AM	**Mechanism of Action of Antibiotic Growth Promoters: Alteration of Immune Response and Intestinal Metabolome** Hyun Lillehoj*, USDA-ARS*		**Itch Alert! There Mite Be A Problem** Robinette Gilbert, *Sanderson Farms, Inc.*
9:45 AM	**Efficacy of Supplemented Bacillus subtilis DSM 17299 in Broiler Chickens Fed Standard and Energy, Protein and Amino Acid- Reduced Diets** John Schleifer, *Chr-Hansen, Inc.*		**I Need You to Call Me Now!** Eric Heskett, *Case Farms*
10:00 AM	**Break**		
Topic	**Avian Influenza**		**Case Report**
Moderator	Blanca Lupiani		Donald Ritter
10:15 AM	**Development of a Novel Avian Influenza Live Virus Vaccine Based on Disruption of M2/M42 Gene Expression** Darrell Kapczynski, *USDA-ARS-Southeast Poultry Research Laboratory*		**Are those Necks Really Crooked?** Danny Magee, *CVM Poultry Research & Diag Lab*
10:30 AM	**Broader Protection from a Recombinant Live Vectored Vaccine than Inactivated Avian Influenza Vaccines in Chickens against Diverse H5 Highly Pathogenic Avian Influenza Viruses** Miria Criado, *USDA*		**Implications of Campylobacter Hepaticus in Organic Laying Facilities** Lisa Tenny, *Kansas State University*
10:45 AM	**Improvement of an NS1-truncated Live Attenuated Influenza Vaccine by Selection of Viral Subpopulations with Enhanced Interferon-inducing Capability** Amir Ghorbani, *The Ohio State University*		**Multiple Barns with High Mortality in a Large Multi-Age Layer Complex** Mohamed El-Gazzar, *Iowa State University*
11:00 AM	**Importance of Vaccine seed strain matching for the control of H9N2 avian influenza** David Suarez, *Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture*		**Analysis of Three Clinical Cases Related to Ionophore Toxicity in Broiler Chickens** Martha Pulido Landinez, *Mississippi State University*

11:15 AM	**Assessing Protection Induced by Maternally Derived Antibodies against Highly Pathogenic Avian Influenza H7N3 Virus** Stivalis Cardenas-Garcia, *University of Georgia*		**Three Times a Charm? Three Different Clinical Presentations of Calcium Toxicity in Meat Type Chickens.** Philip Stayer, *Sanderson Farms*
11:30 AM	**Avian Influenza H6 Virus-like Particles Confers Potent Immunogenicity and Protective Efficacy in Chickens** Hui-Wen Chen, *National Taiwan University*		**Feed Refusal Leading to Spiking Mortality Syndrome in Broiler Chickens** Marie Severyn, *Amick Farms*
11:45 AM	**Mutations in PB1, NP, HA and NA Genes Contribute to Altered Pathogenicity and Viral Shedding of H5N2 Highly Pathogenic Avian Influenza Virus in Chickens and Mallards** Sungsu Youk, *U.S. National Poultry Research Center, ARS-USDA*		**Egg Drop Syndrome (Adenovirus 127) in Layers** Sherrill Davison, *University of Pennsylvania*
12:00 PM	**Lunch Break**		
Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Avian Influenza**		**Case Report**
Moderator	Maricarmen Garcia		Richard Fulton
1:15 PM	**Overlapping Genes of the Avian Influenza Virus M Segment: Role of M2/M42 in Evolution of Highly Pathogenic Avian Influenza Virus** Karen Segovia, *ARS-USDA*		**The Saga Continues: Broiler Variant Reovirus in Eastern North Carolina. Why Did it Happen?** Erin Riley, *Sanderson Farms Inc.*
1:30 PM	**The Molecular Determinants of Antibody Recognition and Antigenic Variations of the HA of H9N2 Viruses** Silvia Carnaccini, *UGA - PDRC*		**Chicks Exhibiting Drunken Behavior** Jean-Pierre Vaillancourt, *University of Montreal*
1:45 PM	**Determining Molecular Markers of Avian Influenza Virus Adaptation in Poultry by Full Genome Sequencing of Different Lineage Viruses** Mary Pantin-Jackwood, *Southeast Poultry Research Lab, US National Poultry Research Center, US Dept. of Agriculture*		**An Outbreak of Very Virulent Infectious Bursal Disease Associated with Severe Mortality in 9-week-old Pullets in California.** H Shivaprasad, *University of California, Davis*
2:00 PM	**In Vivo and In Vitro Differences Between Two H5N8 Highly Pathogenic Avian Influenza Viruses Clade 2.3.4.4** Christina Leyson, *Southeast Poultry Research Laboratory*		**Clinical Investigation of False Laying Syndrome in Commercial Egg Layers** Corine Giroux, *Hickman's Family Farms*
2:15 PM	**Alterations in H5 Highly Pathogenic and Low Pathogenic Recombinant Hemagglutinin Tissue Tropism associated with Increasing Age in Selected Anseriformes and Galliformes Bird Species** Carmen Jerry, *University of Georgia, PDRC*		**Case study - Investigation of Aspergillosis Related Mortality in Commercial Layer Pullets - Diagnose Rare Things Rarely** Albert Payne, *1010 Consulting Group*
2:30 PM	**Challenges in Environmental Sampling and the Use of the VetMAX Gold AI Detection Kit** Len Chappell, *Georgia Poultry Laboratory Network*		**Botulism in Turkeys, Is It More Common Than We Realize?** Kabel Robbins, *Butterball, LLC*
2:45 PM	**Persistence of LPAI in Carcass Composting and Bedding Litter Treated with Acidifier Amendment** Alejandra Figueroa, *University of California, Davis*		**A Case Report of Rotavirus A Hepatitis in California Pigeons** Simone Stoute, *University of California Davis*
3:00 PM	**Break**		

Friday, August 2, 2019

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Epidemiology**		**Vaccinology**
Moderator	Mohamed El Gazzar		Brian Jordan
3:15 PM	**Epidemiological Approaches to Investigate the Determinants of Poultry Diseases and Productivity** Robert Wills, *College of Veterinary Medicine, Mississippi State University*		**New Field Experiences with Dual-Construct Vaccines** Elise Myers, *Merck Animal Health*
3:30 PM	**Understanding How Multiple Production Variables Affect Production Performance Using Statistical Freeware** Dave Fernandez, *AgForte*		**Recombinant HVT/IBD Vaccine As The Sole IBDV Primer In Broiler Breeders: Seroconversion And Progeny Protection Against Challenge With Variant E** Enrique Montiel, *Boehringer Ingelheim*
3:45 PM	**Application of Remote Sensing Technologies to the Epidemiology of Avian Influenza and Other Poultry Diseases** Todd Kelman, *University of California, Davis*		**Protection Against a California 2018 Virulent Newcastle Disease Virus in Pullets by a Vectored HVT-ND-IBD and a Live Vaccine** Alexandra Reilley, *Merck Animal Health*
4:00 PM	**Tactical Epidemiology: Analysis Becomes Action During the Virulent Newcastle Disease Response in California** Annette Jones, *California Department of Food and Agriculture*		**Compatibility of A Recominant HVT-ND Vaccine with Bursaplex to Provide Protections against Velogenic NDV, Virulent Classic IBDV and Virulent MDV Challenges in SPF Birds** Sing Rong, *Zoetis*
4:15 PM	**A Retrospective Study of Variables Associated with the Incidence of Necrotic Enteritis in a No Antibiotics Ever Broiler Complex in the USA** Andrew Bishop, *University of Georgia CVM*		**The Feasibility of the IBDV MB-1 Live Vaccine Strain for In Ovo and Day of Hatch Applications** Ehud Ashash, *Phibro Animal Health Corporation*
4:30 PM	**Epidemiological Investigation of Infectious Bronchitis in Backyard Flocks in Arkansas** Melissa Yates, *Arkansas Livestock & Poultry Commission*		**Evaluation of Three Different Vaccination Programs Against Newcastle Disease in Layers Using ELISA and Hemagglutination Inhibition Test** Elisa Russo, *MSD Animal Health Srl*
4:45 PM	**An Epidemiological Investigation of an Emerging IBD Variant in Broilers in BC, Canada** Babak Sanei, *Zoetis*		**Comparison of Injection Sites for Se Bacterin Vaccine in Commercial Pullets and Layers** Katharine Schlist, *Forsman Farms, Inc.*
Room	Congressional Ballroom AB		
5:00 PM	**Welcome Reception and Poster Session**		

Saturday, August 3, 2019

2019 AAAP Symposium: Investigating Disease and Assessing Productivity Using Epidemiological Tools

8:00 AM	**Welcome**
Room	Renaissance Ballroom West
Moderator	Dave Fernandez
8:10 AM	**Epidemiological Tools in Investigating Disease and Productivity Issues in Poultry – An Overview** Jean-Pierre Vaillancourt, *University of Montreal*
8:45 AM	**Development and Use of Biosecurity Risk Assessments in Commercial Poultry** David Shapiro, *Perdue Farms*
9:20 AM	**Application of Epidemiological Tools in Investigating Disease from Voluntary Disease Databases** Andreia Arruda, *The Ohio State University*
9:50 AM	**Break**
10:00 AM	**Epidemiologic Tools Used in Investigating the 2015 HPAI Outbreak** Brian McCluskey, *APHIS*
10:35 AM	**A Production Veterinarian's Experience Using Epidemiological Tools Investigating Turkey Cellulitis** Brian Wooming, *Cargill Turkeys*
11:10 AM	**Geospatial Analysis and Related Epidemiological Tools in Understanding Common Poultry Diseases** Louise Dufour-Zavala, *Georgia Poultry Laboratory Network*
11:45 AM	**Epidemiological Application to Molecular Techniques in Understanding the Introduction, Spread and Containment of Poultry Diseases** Mark Jackwood, *UGA-PDRC*

Sunday, August 4, 2019

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Wealth of Knowledge**		**Antimicrobial**
Moderator	Hector Cervantes		Michelle Kromm
8:00 AM	**<u>Session Keynote</u>: Economic Perspective of the Poultry Industry** Andrick Payen Diaz De la Vega, *Rabobank RaboResearch Food and Agribusiness Analyst*		**<u>Session Keynote</u>: Quantifying Antimicrobial Use in Poultry Production** Randall Singer, *University of Minnesota*
8:30 AM	**2019 Research Priorities of the American Association of Avian Pathologists** Natalie Armour, *Mississippi State University, CVM, DPPM*		**Organic Acids and Nature Identical Compounds Improve the Efficacy of Conventional Antibiotics Against E. Cecorum** Benedetta Tugnoli, *Vetagro S.p.A.*
8:45 AM	**USDA- National Poultry Improvement Plan Update** Elena Behnke, *USDA- National Poultry Improvement Plan*		**Antimicrobial Effects of Lactic Acid and Bifidobacteria Probiotic Strains and an Enhanced Organic Acid Product Against Salmonella** Chasity Pender, *Biomin America Inc.*
9:00 AM	**Creating a New Cross Commodity Retail Labeled Animal Production Standard Based on the Principles of One Health** G. Donald Ritter, *Mountaire Farms Inc.*		**Evaluation of Dry Hydrogen Peroxide for Reducing Microbial Load in a Commercial Hatchery** Brian Jordan, *The University of Georgia*
9:15 AM	**Surviving the Hurricane of a Lifetime...Again** Becky Tilley, *Butterball*		**Use of Formaldehyde and Hydrogen Peroxide in a Commercial Broiler Hatchery** Sue Hubbard, *Merck Animal Health*
Topic	**Wealth of Knowledge**		**Protozoa/Parasitology**
9:30 AM	**Hurricane Florence: North Carolina Department of Agriculture Response and Impact on NC Poultry Production** Michael Martin, *North Carolina Department of Agriculture and Consumer Services*		**Case Study: Broiler Integrator Performance During Transition to No Antibiotics Ever Production** Linnea Newman, *Merck Animal Health*
9:45 AM	**An Underestimated Tool: 25-Hydroxyvitamin D3 for Intestinal and Bone Health for Broilers Raised without Antibiotics.** Tina Yun-Ting Wang, *DSM*		**Effect of Litter Condition (new litter vs. built-up litter) and Two Anticoccidial Programs (bioshuttle vs. non-bioshuttle) on the Oocyst Concentration in Feces of Broiler Breeder Pullets.** Eric Orozco, *Mississippi State University PRDL*
10:00 AM	**Break**		
Room	Renaissance Ballroom West		
10:15 AM	**Wellness Talk: Student Debt Repayment Strategies** Mike Gergye, *Thales Solutions*		
10:45 AM	**Business Meeting**		
12:00 PM	**Lunch Break**		

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Wealth of Knowledge**		**Protozoa/Parasitology**
Moderator	Yuko Sato		Elise Myers
1:15 PM	**Dinner with Henrietta: Nutritional Issues in the Small Flock** Patricia Wakenell, *Purdue VM: Dept. Comparative Pathobiology*		**Anticoccidial Sensitivity Tests (ASTs): The Do's and Dont's** Hector Cervantes, *Phibro Animal Health*
1:30 PM	**Extension 3.0 in Backyard Poultry Flocks in California** Myrna Cadena, *UC Davis School of Veterinary Medicine*		**Development of a Multi-Locus Sequence Typing (MLST) Scheme for Eimeria maxima** Ruediger Hauck, *Auburn University*
1:45 PM	**Providing Poultry Disease Education in Myanmar** Richard Fulton, *Michigan State University*		**Incorporating Recombinant Eimeria Proteins into Nanoparticles Improves Protective Efficacy Against Avian Coccidiosis** Mark Jenkins, *ARS-USDA*
2:00 PM	**Femoral Head Necrosis (FHN): Incidence and Distribution in the U.S. Broiler Industry** Jaime Ruiz, *Elanco Animal Health*		**Using Polymer Microspheres or Fluorescein to Evaluate Live Coccidiosis Delivery Success Following Commercial Coarse Spray or Gel-Droplet Administration** Ryan Snyder, *University of Guelph*
2:15 PM	**Role of Wooden Breast in Late Mortality in Conventional Broiler Chickens and its Prevalence Prior to Processing** Sesny Gall, *NCSU Veterinary College*		**Characterization and Control by Vaccination of a Pathogenic Eimeria Species Infecting Commercial Chukar Partridge** Jessica Rotolo, *The University of Guelph*
2:30 PM	**What's in the Tracheal Microbiome of a Commercial Broiler Poultry Flock?** Kelly Mulholland, *University of Delaware*		**The Use of a Non-Ionophore Anticoccidial in a Bio-shuttle Program for Breeder Pullets** Takumu Niino, *Zoetis*
2:45 PM	**Enhanced Statistical Process Control Systems for Monitoring and Predicting Live Operations Performance, Plant Performance, and Pathogens** Timothy Buisker, *Smart Data Science Solutions*		**Fifteen Years of Worming Broiler Breeder Pullets/Males** Kelli Jones, *Ceva*
3:00 PM	**Break**		

Sunday, August 4, 2019

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Pathology**		**Immunology**
Moderator	H. Shivaprasad		Juan Rodriguez-Lecompte
3:15 PM	**Broiler Bone Evaluation: What is Normal and Potential Causes of Lameness- Part 1** Suzanne Dougherty, *Pilgrims*		**Susceptibility and Characterization of Anti-viral Innate Immune Responses in Chicken B Cells Infected with Infectious Bursa Diseases Virus and Supplemented with 1,25(OH)2D3** Juan Rodriguez-Lecompte, *Atlantic veterinary College, University of Prince Edward Island*
3:30 PM	**Skeletal Development in a Broiler Chicken Dietary Study: Histopathology Correlates with Clinical Findings** Frederic Hoerr, *Veterinary Diagnostic Pathology, LLC*		**Influence of Feeding Programs on the Innate Immune Responses of Broiler Breeders against Salmonella and E coli** Enrique Montiel, *Boehringer Ingelheim*
3:45 PM	**Myopathic Lesions in Broiler Chicken Heritage Breeds and Red Junglefowl** Hannah Sather, *North Carolina State University College of Veterinary Medicine*		**Innate Immune Responses to Infectious Bronchitis Virus in Tracheal Organ Cultures from MHC Congenic Chicken Lines** Ana Paula Da Silva, *UC Davis*
4:00 PM	**I See Liver Spots!** Claude Hebron, *Prestage Farms*		**Establishing Reference Ranges for Blood Gas and Blood Chemistry for Commercially Grown Pullets in North Georgia using the i-STAT ® Alinity v Handheld Clinical Analyzer** Randi Clark, *Poultry Diagnostic and Research Center*
Topic	**Food Safety**		**Immunology**
Moderator	Robin Gilbert		Juan Rodriguez-Lecompte
4:15 PM	**Salmonella Enteritidis Invasion of Internal Organs and Contamination of Eggs from Experimentally Infected Laying Hens of Four Commercial Genetic Lines** Richard Gast, *U.S.National Poultry Research Center, USDA-ARS*		**Recombinant Avian Interferons as Long Lasting Adjuvants in Live IBV Vaccines.** Jose Cano, *Universidad Nacional Autonoma de Mexico*
4:30 PM	**Effect of Yeast Derived Feed Additive on Salmonella and Antibiotic Resistance** Michelle Kromm, *Jennie-O Turkey Store*		**Evaluation Of Humoral And Cellular Immune Responses Of Broiler Breeders Following Vaccination With Inactivated FAdV Vaccine Adjuvanted With Emulsigen-D Or Cpg-ODN** Ashish Gupta, *Western College of Veterinary Medicine, University of Saskatchewan*
4:45 PM			**Correlating Breeder Flock Serology with Maternal Antibody Titers in Poults** Katie Stumvoll, *Jennie-O Turkey Store*
5:00 PM	**Adjorn**		

Monday, August 5, 2019

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Food Safety**		**Bacteriology**
Moderator	John Schleifer		Naola Ferguson-Noel
8:00 AM	**<u>Session Keynote</u>: Sex and the Single Salmonella: What We Don't Know Hurts Us** Jean Guard, *USDA*		**<u>Session Keynote</u>: Evaluation of the Impact of Mycoplasma synoviae Infection on the Efficacy of Infectious Laryngotracheitis CEO Vaccination in Broiler Chickens** Naola Ferguson-Noel, *University of Georgia*
8:30 AM	**Immunization with Live Salmonella Vaccine, Programs in Broiler Chickens and Resulting Impact in Plant Samples** Robert Evans, *Elanco*		**Field Trial of Live Mycoplasma synoviae (MS) Vaccine in Area of High MS Prevalence** Myeongseob Kim, *Boehringer Ingelheim*
8:45 AM	**Live Salmonella Typhimurium Vaccination Lowers the Load of Various Salmonella Serogroups at Different Challenge Ages in Broilers** Manuel Da Costa, *Zoetis*		**Development and Validation of Mycoplasma iowae (MI) Core Genome Multilocus Sequence Typing (cgMLST) Scheme** Mostafa Ghanem, *The Ohio State University*
9:00 AM	**Use of Salmonella Vaccines in Commercial Turkeys for Pre-Harvest Reduction in Salmonella Load at Processing** Megan Lighty, *Jennie-O Turkey Store*		**Clinical 6/85 Mycoplasma Gallisepticum Vaccine Infection in Pre-Production Turkey Breeders** Judith LaBounty, *Rembrandt Foods*
9:15 AM	**Most Effective Form of Vaccination Application Using a Live Attenuated Salmonella Typhiumurium Vaccine** Jolene Tourville, *Jennie-O Turkey Store*		**Campylobacter and Microbiome Interactions in Broiler Litter: a Matched Case Control Study** R. Chacin-Valeris, *University of MN*
9:30 AM	**Feed and Drinking Water Delivered Chitosan-Subunit Salmonella Nanovaccine for Poultry** Sankar Renu, *The Ohio State Univsersity*		**Avian Pathogenic E. Coli Genotypes Across the US Broiler and Turkey Industries.** Daniel Karunakaran, *Arm and Hammer Animal Nutrition*
9:45 AM	**Evaluation of Long-Term Immunity and Protection against Salmonella spp by an Orally Administrated Subunit Vaccine** Sherry Layton, *Vetanco/BV Science*		**Characterization of Omphalitis Causing Extra-Intestinal Pathogenic Escherichia Coli** Martine Boulianne, *Université de Montréal*
10:00 AM	**Break**		

Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Reovirus**		**Bacteriology**
Moderator	Kalen Cookson		Patricia Wakenell
10:15 AM	**Genotypic Diversity Among Avian Reovirus Field Isolates** Holly Sellers, *The University of Georgia*		**The Role of Enterococcus Faecalis in the Modulation of Avian Pathogenic E. coli Infections of Poultry** Grayson Walker, *North Carolina State University CVM*
10:30 AM	**Evaluation of the Pathogenicity of Recent Reovirus Field Isolates in Genotypes 2, 4 and 6 in Commercial Broilers** Angela Stewart, *Poultry Diagnostic and Research Center - University of Georgia*		**Attempts to Reproduce Focal Duodenal Necrosis by Experimental Infection of Chickens with Clostridium Perfringens Isolates and Duodenal Homogenates** Monique Franca, *UGA*
10:45 AM	**Cold Adaptation and Evaluation of an Avian Reovirus Genotype One Variant** William Dawe, *University of Georgia*		**16s Phylogenomic Analysis of Environmental and Tissue Microbiota for Correlating Management Strategies Related to Incidence of Clostridial Dermatitis in Turkeys** Yuko Sato, *Iowa State University*
11:00 AM	**Horizontal Transmission of Reovirus and Age-Associated Susceptibility to Development of Viral Arthritis/Tenosynovitis** Milos Markis, *AviServe LLC*		**Ornithobacterium Rhinotracheale (ORT): Never Ending Story** Hafez Mohamed Hafez, *Institute of Poultry Diseases Free University Berlin*
11:15 AM	**My Experience with Laboratory Diagnosis of Reovirus Tenosynovitis** Tahseen Abdul-Aziz, *Rollins Animal Disease Diagnostic Laboratory*		**In Vitro and In Vivo Evaluation of Probiotic Supplementation and its Effect on Performance and Immune Response in Campylobacter-Challenged Broilers** Mohamad Mortada, *University of Georgia*
Room	Renaissance Ballroom West		
11:30 AM	**History Lecture: Pullorum Disease: Evolution of the Eradication Strategy** K.V. Nagaraja, *University of Minnesota*		
12:00 PM	**Lunch Break**		
Topic	**Virology**		**Diagnostics**
Moderator	Daral Jackwood		Louise Dufour-Zavala
1:15 PM	**Immune Responses Induced in Chickens by a Genetically More Homogeneous Infectious Bronchitis Virus Vaccine** Haroldo Toro, *Auburn University*		**Evaluation of Sample Collection Devices for Recovering Poultry Respiratory Viruses from the Environment** Erica Spackman, *SEPRL-USDA-ARS*
1:30 PM	**Broiler Study Evaluating Arkansas IBV Protection Achieved By Various Heterologous IB Vaccine Combinations** Kalen Cookson, *Zoetis*		**Re-emerging Turkey Arthritis Reovirus - Diagnostic Strategies** Sunil Mor, *University of Minnesota*
1:45 PM	**False Layer Syndrome Caused by Infectious Bronchitis Virus, Genetic Characterization and Pathobiology Insights** Rodrigo Gallardo, *University of California, Davis*		**Avian Reoviruses: Molecular Characterization In California** Sofia Egana, *University of California, Davis*
2:00 PM	**Use of Different NDV ELISA Kits for the Evaluation of the Antibody Response at Different Ages Elicited by a Recombinant Newcastle Disease Vaccine (HVT+ND) Applied In Ovo in Both Specific Pathogen Free (SPF) Birds and Commercial Broilers** Claudia Osorio, *Boehringer-ingelheim*		**Molecular Characterisation of Chicken Arthritis Reoviruses Circulating in Israel, Germany and the United States** Rahul Kumar, *University of Minnesota*

2:15 PM	**Host Adaptation Affects the Transmission of Virulent Newcastle Disease Viruses in Chickens** Helena Lage Ferreira, *US National Poultry Research Center, SEPRL*		**Developing and Application of Real Time PCR Protocols to Differentiate Mycoplasma synoviae Vaccine Strains** Mohammadreza Ehsan, *University of Georgia*
2:30 PM	**Molecular-Epidemiological and Evolution Study of the Newcastle Disease Virus Causing Outbreaks in California in 2018** Kiril Dimitrov, *USDA/ARS/USNPRC/SEPRL*		**Infectious Bronchitis in Immunologically Naive Breeding Chickens and Their Progeny** Corissa Steimling, *The Pennsylvania State University Animal Diagnostic Laboratory*
2:45 PM	**Evaluation of Protection against AL-2 like Infectious Bursal Diseases Virus using a Dual Recombinant HVT-ND-IBDV Vaccine Along and in Combination with a Live 89/03 and Two Plaque Intermediate Standard Strains of IBDV Vaccine** Andres Montoya, *Merck Animal Health*		**Infectious Laryngotracheitis Prevalence in California Backyard Chicken Flocks and Strain Differentiation by ICP4 Sequencing, 2007-2017.** Julia Blakey, *California Animal Health and Food Safety Lab- UC Davis*
3:00 PM	**Break**		
Room	Renaissance Ballroom East		Renaissance Ballroom West
Topic	**Virology**		**Management**
Moderator	Kristen Roza-Sutherland		Becky Tilley
3:15 PM	**Study of pathogenesis of Infectious Bursal Disease Virus (IBDV): Comparison between a South American Variant and a Classic Strain** Ariel Vagnozzi, *INTA*		**Evaluation of Enrichments for Meat-type Chickens** Katherine Barger, *Cobb Vantress Inc*
3:30 PM	**Cell-mediated Immune Responses in the Eye Associated Lymphoid Tissues of Chickens after Vaccination or Infection with Infectious Laryngotracheitis Virus (ILTV)** Maricarmen Garcia, *The University of Georgia, Athens GA*		**Effects of Sanitation Water Treatments During Broiler Growth-Out** Ricardo Munoz, *Neogen*
3:45 PM	**Interpretation of Real-time PCR Data from Clinical Specimens Suspected for Vaccinal Laryngotracheitis Virus Infection.** Arun Kulkarni, *Georgia Poultry Laboratory*		**What are You Doing to Day-Old Poults** Ben Wileman, *Select Genetics*
4:00 PM	**Efficacy of Innovax-ILT (recombinant HVT-LT) Vaccine when Administered Alone or in Combination with a Chicken Embryo Origin (CEO) Vaccine** Daniel Maekawa Maeda, *University of Georgia*		**Implementation and Evaluation of a Biosecurity Audit Tool Within a Large Turkey Company** Molly Parker, *Butterball, LLC*
4:15 PM	**The Role of Vaccination on Transmission of Marek's Disease Virus in Poultry** John Dunn, *USDA-ARS-ADOL*		**Impact of Downtime on Performance and Livability in Commercial Turkey Flocks** Elise Gerken, *Jennie-O Turkey Store*
4:30 PM	**Are Birds Expressing Cas9 and Guide RNAs against Marek's Disease Virus (MDV) Resistant to MDV Challenge?** Karel Schat, *Cornell University*		**Pullet Vaccination Evaluations: The Good, the Bad, and the Ugly** Timothy Cummings, *Zoetis*
4:45 PM	**Next Generation Sequencing (NGS) of Haemorrhagic Enteritis Virus (HEV) Obtained from Spleens of Turkeys Raised in Western Canada** Victor Palomino-Tapia, *University of Calgary*		**Comparison of the Circadian Rythm of Broiler Brreders and Commercial Layers During Lay on Different Biochemical Parameters with an Emphasis on Shell Formation** Daniel Venne, *Couvoir Scott Ltée*
5:00 PM	**Adjorn**		

Posters

Poster numbers are listed below. Posters should be put up in Congressional Hall AB by Friday, August 2nd 8:00 AM and taken down by Monday, August 5th 12:00 PM.

Antimicrobial

1. **Reducing Microbial Load on Hatching Eggs Using a Dry Hydrogen Peroxide Gas System**
   Julia McElreath, *University of Georgia*

2. **Efficacy of Sodium Formate (Amasil NA), Monobutyrin, Other Glycerides, and Glycerol (SiloHealth 104),) and Bacitracin Methylene Disalicylate (BMD) on Intestinal and Processed Parts Bacteria Count when fed to Broiler Chickens Challenged with Mild Coccidia**
   Michael Coelho, *BASF Corp*

3. **Are You Ready for an FDA Inspection? Ensuring a Valid VCPR for Poultry Prescriptions and Protocols**
   Steven Clark, *Devenish Nutrition, LLC*

4. **Prevalence and Antimicrobial Resistance of Fecal Commensal E. coli and Salmonella Isolates Obtained from Ontario Small Poultry Flocks**
   Csaba Varga, *Ontario Ministry of Agriculture Food and Rural Affairs*

5. **Antimicrobial Resistance Patterns in Campylobacter Species Isolated From Poultry Production Facilities in Alberta**
   Karen Liljebjelke, *University of Calgary*

Avian Influenza

6. **Risk Assessment of Avian Influenza Virus Dissemination in Duck Farms in France**
   Kateri Bertran, *ENVT, University of Toulouse*

7. **Prevention and Control of Avian Influenza Through Biosecurity, Surveillance, Early Detection, and Rapid Response: It Is Easier Said Than Done**
   Nathaniel Tablante, *University of Maryland College Park*

8. **Outbreaks of Low Pathogenic Avian Influenza H7N3 in Turkeys in California.**
   H Shivaprasad, *University of California, Davis*

9. **Vaccine Protection of Commercial Broilers Vaccinated with an Avian Influenza Vector Vaccine rHVT-H5 and an Inactivated H5N2 Vaccine and Challenged with the Mexican H5N2 Highly Pathogenic Avian Influenza Virus (HPAIV)**
   Luiz Sesti, *Ceva Animal Health*

10. **Chimeric Virus-Like Particle Vaccine Elicits Strong Antibody Response against Different Clades of H5 Highly Pathogenic Avian Influenza Viruses**
    Dong-Hun Lee, *Department of Pathobiology & Veterinary Science, University of Connecticut*

11. **Genetic Characterization and Transmissibility of the First H9N2 Avian Influenza Virus in Indigenous Chickens from Kenyan Live Bird Markets**
    Henry Kariithi, *USDA-ARS*

12. **Pathogenicity and Transmission of Clade 2.3.4.4 H5Nx Highly Pathogenic Avian Influenza Viruses in Chickens.**
    Junghoon Kwon, *U.S. National Poultry Research Center*

13. **Development and Comparison of Two Primary Chicken Tracheal Cell Culture Systems for the Study of Avian Influenza Virus Infection**
    Emily Aston, *University of California-Davis*

14. **Extended Laboratory Exercise on Surge Capacity of Highly Pathogenic Avian Influenza (HPAI) Virus Testing**
    Stephanie Forrester, *Georgia Poultry Laboratory Network*

Impact of Immune Pressure on Mutations of Highly Pathogenic Avian Influenza Virus in Vaccinated Poultry
Darrell Kapczynski, *USDA-ARS-Southeast Poultry Research Laboratory, Athens, Ga*

Bacteriology

15. **Characterization of the duodenal microbiota in egg layers with Focal Duodenal Necrosis using next generation sequencing**
Monique Franca, *UGA*

16. **Molecular Characterization of Avian Pathogenic E. coli (APEC) from Turkey Cellulitis**
Catherine Logue, *University of Georgia*

17. **Complete Genome Sequence of Mycoplasma iowae (MI) type strain 695**
Mostafa Ghanem, *The Ohio State University*

18. **Outbreaks of infectious endocarditis in mule duck: epidemiological study and potential etiological role of Streptococcus pluranimalium**
Jean-Luc Guerin, *ENVT, University of Toulouse*

19. **Pathogenecity of Avibacterium paragallinarum Isolates with Different Nicotinamide Adenine Dinucleotide Requirements of South Korea**
Ok-Mi Jeong, *Animal and Plant Quarantine Agency*

20. **Effect of a Feed Incorporated Phytobiotic on Intestinal Lesions in Poultry Challenged with Clostridium perfringens**
Sherry Layton, *Vetanco BV Science*

21. **Mycobacterial Infection in a Pet Zebra Finch**
Kristen Hill-Thimmesch, *Purdue University*

22. **Identification of Gallibacterium Anatis in Egg Producing Hens Using the PCR Test in Los Altos de Jalisco, Mexico**
Alejandro Hori, *Boehringer Ingelheim Animal Health*

Case Reports

23. **Chicks Exhibiting Drunken Behavior**
Jean-Pierre Vaillancourt, *University of Montreal*

24. **Mycotoxins Toxicity in Laying Hens**
Andres Rodriguez-Avila, *BioARA SA*

25. **Chronic Mycosis in a Backyard Hen**
Laura Morman, *Purdue University Indiana Animal Disease Diagnostic Lab*

Diagnostics

26. **A Summary of Epidemic Pattern of Increased Infectious Bronchitis Virus Isolations in Broiler and Layer Chickens in Pennsylvania**
Huaguang Lu, *The Pennsylvania State University*

27. **Identification and Lineage Typing of Infectious bronchitis virus in clinical samples by real-time MinION Nanopore sequencing**
Salman Latif Butt, *University of Georgia, Athens*

28. **Poultry Vaccines: Innovative Serological Assays for Diagnosis and Vaccination Monitoring for H5, H7 and H9 Avian Influenza A**
Laure Papaix, *IDvet*

29. **Validation of Two New Real-Time Avian Influenza PCR Kits**
Gwenllyan Slacum, *BioChek USA Corp.*

30. **Optimization Of The Protocol For Detection of Turkey Arthritis Reovirus by RT-PCR**
Robert Porter, *Univ of Minnesota*

31. **Use of an Infectious Laryngotracheitis Virus gB Protein Elisa kit to Assess the Serological Response to Vaccination**
Fernando Lozano, *CEVA ANIMAL HEALTH*

32. **MinION sequencing to genotype US strains of infectious laryngotracheitis virus**
Stephen Spatz, *US National Poultry Research Center, Agricultural Research Service, United States Department of Ag*

33. **Isolation, identification and genomic characterizations of two different genotypes of avian paramyxoviruses 1 isolated from live bird markets in Tanzania in 2012**
Iryna Goraichuk, *USDA-ARS-USNPRS-SEPRL*

34. **Expression and characterization of chicken anemia virus proteins as ELISA antigens**
Linda Michel, *The Ohio State University*

35. **A Retrospective Study (2016-2017) of Neoplastic Diseases Diagnosed in Backyard Chickens Submitted to the Alabama Department of Agriculture and Industries Veterinary Diagnostic Laboratory System**
Erfan Chowdhury, *Alabama Veterinary Diagnostic Laboratory System*

36. **In vivo testing of a qPCR for the specific detection of a new MDV serotype-1 vaccine (RN1250 strain) administered at day-old chicks**
Andrea Delvecchio, *Boehringer Ingelheim AH*

37. **Evaluation of an Easy and Rapid Detection of Avian Leukosis Virus Subgroup J (ALV-J) by Fully Automated POCKIT™ Central PCR System**
Keat Fu, *Aviagen Inc.*

38. **A Portable Microdevice for Size-based Virus Enrichment**
Huaguang Lu, *The Pennsylvania State University*

39. **Isolation and TEM examination of unknown duck viruses**
Huaguang Lu, *The Pennsylvania State University*

40. **Validation of real time PCR reagents to identify Salmonella spp. DNA in enriched cultures**
Jantina De Vylder, *BioChek*

41. **A Comprehensive Comparison of Multiple MG/MS Real Time PCR Assays**
Heather Failyer, *Georgia Poultry Laboratory Network*

42. **Diagnostic Service for Avian Pathogenic Escherichia coli (APEC) Identification in Georgia: Comparison of Gene Profiles with Tissue of Isolation.**
Nicolle Barbieri, *University of Georgia*

45. **Next-generation random Sequencing for Identification and Characterization of Infectious Agents in broilers with Hypoglycemia and Spiking Mortality Syndrome**
Iryna Goraichuk, *USDA/ARS/SEPRL*

46. **Why a Quality Managment System is Valuable in a Poultry Laboratory**
Brenda Glidewell, *GA Poultry Lab*

47. **Isolation of Salmonella sp. in Poultry Tissue Samples using the MSRV Method**
Bridgeth Flores, *GA Poultry Lab*

48. **Development of Multiplex Polymerase Chain Reaction for Detection of Avian Leukosis Virus, Reticuloendotheliosis Virus, Marek's Disease Virus and Chicken Infectious Anemia Virus in Korean Native Chicken**
You-Chan Bae*, Animal and Plant Quarantine Agency*

Enteric Health

49. **The effect of dietary supplementation of several Bacillus strains on growth performance and gut health in mixed coccidiosis infection in chickens**
Atul Chaudhari, *USDA-ARS*

Food Safety

50. **The Effectiveness of a Feed Grade Sodium Formate in Feed or Drinking Water in Reducing Salmonella heidelberg Colonization of Broilers**
Charles Hofacre, *Southern Poultry Research Group, Inc.*

51. **Estimating Salmonella Numbers to the Processing Plant: a Correlation of Salmonella Enumeration of Bootsocks, Ceca and Carcass Rinses from Broiler Chickens**
Charles Hofacre, *Southern Poultry Research Group, Inc.*

52. **Salmonella enterica Subsp. enterica Serovar Typhimurium Weakens Avian Blood Macrophage-like Monocytes Functions In Vitro**
Elya Abbaszadeh, *The University of Tehran*

53. **Salmonella Field Isolates: A Retrospective Analysis of Salmonella Serotypes Association with Common Field Isolation Media of Poultry Flocks in the Southern United States**
Carolyn Miller, *Consultant*

54. **What to do with that Salmonella Culture over the Weekend**
Doug Waltman, *Georgia Poultry Laboratory Network*

55. **Comparing Salmonella spp. Isolated Based on Organ Type**
Kathryn Burden, *GA Poultry Lab*

Immunology

56. **Development of Sandwich ELISA for Differential Detection of Chicken Heterodimeric Cytokines, Interleukin-12 and -23**
Woohyun Kim, *USDA-ARS*

57. **Development of novel monoclonal antibodies against chicken interleukin- 4 and alternative activation of macrophage-like cells in chickens**
Atul Chaudhari, *USDA-ARS*

58. **Development of Poultry-Specific Immune Reagents**
Hyun Lillehoj, *Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, United States*

60. **Clostridium perfringens induces IL-10 secretion in chicken intestinal epithelial cells and necrotic enteritis broiler chicken**
YOUNGSUB LEE, *USDA-ARS*

61. **Comparison of toll-like receptor (TLR) 3 mediated immune responses in wild-type and TLR3 knockout chicken and quail fibroblast cell lines**
Mahesh K.C., *The Ohio State University*

62. **Expression and Characterization of Chicken Perforin and Granzyme A**
Charles Li, *Beltsville Agricultural Research Center / ARS / USDA*

63. **Effect of In Ovo Vaccination with HVT on IFN-gamma and TLR-3 Transcripts in the Spleen and Lung of Chicken Embryos**
Allison Boone, *North Carolina State University, College of Veterinary Medicine and North Carolina Veterinary Diagno*

64. **Identification of nucleotide-binding oligomerization domain like receptor pyrin domain containing 3 inflammasome in chickens**
Ching Ching Wu, *School of Veterianry Medicine, National Taiwan University, Taipei, Taiwan*

65. **Development Of The Immune System In Broiler Breeders: Impact Of IBDV Vaccination and Feeding Programs**
Enrique Montiel, *Boehringer Ingelheim*

66. **Correlation Between the Level of Maternal Antibodies in Broilers Against Chicken Anemia Virus and Infectious Bursal Disease Virus with Respect to the Time of Egg Storage and the Age of the Breeders**
Xanat Alicia Roa Garcia, *Boehringer Ingelheim Animal Health Mexico*

67. **Comparison of the Serological Response of Three Tetravalent Commercial Inactivated Vaccines in Breeders**
Bruno Garcia, *San Marcos University*

68. **Effects of glycinated zinc on host immune response to necrotic enteritis in broilers**
Theros Ng, *University of Georgia*

Marek's Disease Virus

69. **The effects of CVI988 Marek's disease vaccine in eliciting transcription of IFN-gamma and TLR-3 in turkey embryos**
Jewell Bremer, *North Carolina State University CVM*

70. **Effect of Challenge with Oncogenic Marek's Disease Virus on the Replication of Marek's Disease Vaccines**
Rachel Reese, *North Carolina State University CVM*

71. **Study of the efficacy and replication of recombinant vector vaccines using herpesvirus of turkey (rHVT) when combined with other Marek's disease vaccines**
Aneg Lucia Cortes, *North Carolina State University, CVM*

72. **Role of Meq-vIL8 in the Pathogenesis of Marek's Disease Virus**
Blanca Lupiani, *Texas A&M University*

Parasitology

73. **Fenbendazole Resistance in the Turkey Nematode, Ascaridia dissimilis**
James Collins, *University of Georgia, Dept. of Infectious Diseases*

74. **Eimeria tenella Elongation Factor-1a (EF-1a) co-administered with chicken IL-7 (chIL-7) DNA vaccine emulsified in Montanide Gel 01 adjuvant enhanced immune response to E. acervulina infection in broiler chickens.**
Alfredo Panebra, *USDA*

75. **Evaluation of a Potential Universal Subunit Coccidosis Vaccine to Protect Against Mixed Eimeria spp Challenge**
Sherry Layton, *Vetanco/BV Science*

76. **Histomoniasis in broiler breeders in Peru**
Nelly Cribillero, *San Marcos University*

77. **Populations of Eimeria tenella Express Resistance to Commonly Used Anticoccidial Drugs in Southern Nigeria**
Agatha Ojimelukwe, *University of Port Harcort*

Pathology

78. **Mycotoxicosis by T-2 Toxin in Broiler Breeders in Peru**
Eliana Icochea, *UNMSM*

79. **A Cervical Mass in an Adult Backyard Hen**
Kristen Hill-Thimmesch, *Purdue University*

80. **Description of round cell neoplasia in psittacine birds to characterize diagnostic features**
Daniel Gibson, *The University of Guelph*

81. **Neoplasia in Small Poultry Flocks in Ontario**
Leonardo Susta, *University of Guelph*

82. **Histologic and Histomorphometric Evaluations of Disarticulation-Associated Femoral Head Separation in Clinically Normal Broilers: Documentation of Underlying Predisposing Cartilage Abnormalities**
Floyd Wilson, *MVRDL*

83. **Production Trial Analysis in Commercial Layers Using Mortality Survey Analytics and Cumulative Mortality**
Frederic Hoerr, *Veterinary Diagnostic Pathology, LLC*

Vaccinology

84. **Protection Conferred by Infectious Bronchitis Virus Spike Ectodomain**
Ramon Zegpi, *Auburn University*

85. **Oral DNA vaccination with vaccine encoding turkey coronaviral spike protein containing neutralizing epitope delivered by attenuated Salmonella with the boost of spike protein**
Tsang Long Lin, *Purdue University, CVM*

86. **Characterization of mode of action of MB-1 – a live hatchery vaccine against Gumboro disease.**
Ehud Ashash, *Phibro Animal Health Corporation*

87. **Construction and Efficacy of A Recombinant HVT-ND Vaccine against NDV and MDV Challenges in SPF and NDV Challenge in Broiler Birds**
Sing Rong, *Zoetis*

88. **Onset of Immunity of A Recombinant HVT-ND against of a Velogenic NDV Challenge in SPF Birds**
Sing Rong, *Zoetis*

89. **Evaluation of attenuated fowl adenovirus vaccines against Inclusion Body Hepatitis in chicken**
Van Dam Lai, *Chungbuk National University, CVM*

90. **Compatibility evaluation of two Fowl Pox Vectorized Vaccines against the Avian Influenza Virus H7N3 and H5N2 administered simultaneously.**
Lilia Castellanos, *Boehringer Ingelheim Animal Health Mexico*

91. **A Fowlpox-vectored H7 Influenza Vaccine to Help Combat H7N3 Avian Influenza Virus Circulating in Mexico.**
Justin Widener, *Boehringer Ingelheim Animal Health*

92. **Seroconversion and Vaccine Take Monitoring in SPF Birds Vaccinated, Alone or Combination, with Different HVT Vector Vaccines Against Newcastle or Avian Influenza (H5 or H7) Viruses and an Inactivated Reverse Genetics Vaccine Against the Avian Influenza Vi**
Luiz Sesti, *Ceva Animal Health*

94. **Assesment of a Novel 4 Way Inactivated Vaccine for Broiler Breeders.**
Francisco Perozo, *Merial*

95. **Verification of in Ovo Vaccination Using Next Generation Sequencing and a Traffic Light- Style Scoring System**
Elise Myers, *Merck Animal Health*

96. **Optimization of antibody avidity index estimation using logistical regression models**
Ramon Zegpi, *Auburn University*

97. **Field safety of in ovo and subcutaneous administration of Innovax-ND-IBD (HVT-ND-IBD) vaccine in commercial Broilers**
Matthew Stock,

138. **Development of a Genotype-Matched Vaccine Against Virulent Newcastle Disease Virus**
Dana Goldenberg, *USDA ARS*

Virology

98. **IBV DMV/1639/11 causing cystic oviducts in egg layers: Prevalence and risk factors in Eastern Canada laying flocks**
Eric Parent, *Université de Montréal, Saint-Hyacinthe, Canada*

99. **Virus neutralization of the Canada/17-099269/17 strain of Infectious Bronchitis Virus using antisera to known vaccine strains**
Andrew Bishop, *University of Georgia PDRC*

100. **Broiler Study Evaluating Protection Against A Contemporary DMV/1639 Isolate By A Commercial GA08 Vaccine Either By Itself Or In Combination With Other IB Vaccines**
Kalen Cookson, *Zoetis*

101. **Genetic and Pathologic Characterization of Recombinant TC07-2-like and Q1-like Infectious Bronchitis Viruses in Korea**
So Youn Youn, *Animal and Plant Quarantine Agency*

102. **Intestinal tropism of an IBV isolate is not explained by spike protein binding specificity**
Saiada Farjana, *Auburn University*

103. **Serology and Molecular Diagnostics as Tools to Assess Losses Induced by Infectious Bronchitis (IB) in Broiler Farms**
Jorge Chacón, *Ceva Animal Health*

104. **Control of Production Losses in Commercial Layer Flocks Infected with the BR-I Variant Strain of Infectious Bronchitis Virus in Brazil**
Jorge Chacón, *Ceva Animal Health*

105. **Infectious Bronchitis Virus (IBV) in Peru: a 10-year molecular epidemiological survey (2009 to 2018)**
Claudia Carranza, *USP / Ceva Animal Health*

106. **Economic Estimation of Direct Disease Cost of an IBV Outbreak in a Grandparent Flock in Brazil**
Claudia Carranza, *USP / Ceva Animal Health*

107. **Persistence of Infectious Bronchitis and Newcastle Disease Vaccine virus by In Situ Hybridization**
Alejandro Banda, *Mississippi State University*

108. **Seroconversion of Two ND Vaccination Programs**
Bruno Garcia, *San Marcos University*

109. **Virulent Newcastle disease virus efficiently replicates in chicken feathers and is readily identified by rRT-PCR and virus isolation**
Kiril Dimitrov, *USDA/ARS/USNPRC/SEPRL*

110. **Evaluation of Factors Influencing Marek's Disease Vaccine Titer Variability**
Blanca Lopez de Juan Abad, *North Carolina State University*

111. **Vaccinal efficacy of Gallid alphaherpesvirus 3 strain 301B/1 from BAC clone against Marek's disease virus challenge**
Taejoong Kim, *USDA-ARS-USNPRC*

112. **Use of HVT vector to express protective antigens from important viral pathogens**
Sanjay Reddy, *Texas A & M University*

113. **Current Infectious Bursal Disease Virus Circulation in Mexican Poultry. A Molecular Survey**
Francisco Rios-Cambre, *MSD Salud Animal Mexico*

114. **New Introduction of a Very Virulent Infectious Bursal Disease Virus in the USA**
Daral Jackwood, *The Ohio State University*

115. **Towards the Construction of Sigma C epitope-based Vaccine for Avian Reoviruses**
Joseph Giambrone, *Auburn University*

116. **Pathogenicity of a variant Reovirus isolate close to Classical strains obtained from Clinical case of Viral Arthritis in Northwest Canada.**
Victor Palomino-Tapia, *University of Calgary*

117. **Characterizing the etiological agent of guinea fowl viral pancreatitis**
Jean-Luc Guerin, *ENVT, University of Toulouse*

118. **Defining the Host Range of Aquatic Bird Bornavirus through In Vitro Replication Kinetics and Lesion Development In Ovo**
Alex Leacy, *University of Guelph*

119. **A Preliminary Survey of Hemagglutinating Viruses from Penguins, Great Grebes and Petrels in the Coast of the District of Pinamar, Argentina.**
Celina Buscaglia, *Comision de Investigaciones Cientificas de la Provincia de Buenos Aires*

120. **Evaluation of Infectious Bronchitis Virus (IBV) Infection in Broilers Fed Availa®Zn**
Brian Ladman, *University of Delaware*

Wealth of Knowledge

121. **Non-biological factors impacting efficacy of Direct Fed Microbials in commercial poultry production**
James Barton, *Adisseo*

122. **Peptidoglycan: An issue in chicken intestinal tract that has been overlooked.**
Tina Yun-Ting Wang, *DSM*

123. **Toward Understanding the Respiratory Microbiota of Poultry**
Chang-Won Lee, *Food Animal Health Research Program, The Ohio State University*

124. **Field Experiences in Preventing Bacterial Bone Disease in Broiler Chickens**
Marie Severyn, *Amick Farms*

125. **Big Data in Poultry Production: Harnessing Information Regardless of Scale**
Albert Payne, *Simple Ag Solutions*

126. **Using Telemedecine for Training and for Collaborative Initiatives in the Field**
Jean-Pierre Vaillancourt, *University of Montreal*

127. **Incorporating the New NPIP SubPart J into the State's Plan.**
Douglas Anderson, *GPLN*

128. **Industry Strategies for Inclusion Body Hepatitis Control in Peru.**
Francisco Perozo, *Merial*

129. **Poultry Health Management Schools: Continuing Education For the Poultry Health Professional**
Robert Porter, *Univ of Minnesota*

130. **The effects of downtime on key chicken production parameters**
Timothy Buisker, *Smart Data Science Solutions*

131. **Characterization of the Demographics and Prevalence of Poultry Pathogens in Ontario, Canada Small Poultry Flocks**
Marina Brash, *Animal Health Laboratory*

132. **Causes of Morbidity and Mortality in Small Flocks in Ontario**
Leonardo Susta, *University of Guelph*

133. **Review and statistical analysis of trends in common diseases of pet/hobby chickens submitted to the Georgia Poultry Lab Network**
Christopher Gaudette, *North Carolina State University CVM*

134. **Mycotoxin Prevalence in the 2018 Latin American Corn Crop**
Eduardo Vicuna, *BIOMIN*

135. **Megabacteriosis, Macrorhabdus ornithogaster and Mycobacterium genavense in a Zoo Budgerigar, Melopsittacus undulatus, Flock**
Richard Fulton, *Michigan State University*

136. **A Historical Snapshot of Turkey Production in the USA**
Joan Schrader, *Poultry Veterinarian & Consultant*

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Table of Contents

Oral Presentations

Symposium
Enteric Health
Avian Influenza
Epidemiology
Wealth of Knowledge
Pathology
Food Safety
Reovirus
Virology
Case Reports
Vaccinology
Antimicrobial
Protozoa/Parasitology
Immunology
Bacteriology
Diagnostics
Management

Posters

Antimicrobial
Avian Influenza
Bacteriology
Diagnostics
Case Reports
Enteric Health
Food Safety
Immunology
Marek's Disease Virus
Parasitology
Pathology
Vaccinology
Virology
Wealth of Knowledge

2019 AAAP Symposium: Investigating Disease and Assessing Productivity Using Epidemiological Tools

Epidemiological tools in investigating disease and productivity issues in poultry – An overview

Jean-Pierre Vaillancourt

University of Montreal

Today's challenges, including reducing antibiotic usage and antibiotic-free production, require a multifaceted approach to problem solving in poultry medicine. Epidemiology offers different tools facilitating infectious and non-infectious disease investigations and management, including the benefit-cost analysis of disease control programs. They include various study designs (observational: cohort, case-control, cross-sectional; experimental: randomized controlled trials, quasi-experimental designs, interventional), and even theoretical epidemiology using mathematical models to explain and examine aspects of disease behavior with simulation models allowing the exploration of different scenarios. Furthermore, several measurement tools now exist (surveys and data management softwares facilitating the calculation of proportions, ratios, rates, prevalence, incidence, health and production indices, odds and risk ratios, confidence intervals, etc.). Analytical techniques are also very useful and many novel approaches are being applied in veterinary medicine besides classic descriptive and analytical techniques such as univariate and multivariable regression. The need to explore new analytical methods has been driven by the increasing availability and complexity of disease (e.g., molecular) and environmental data ("big data"). Approaches such as multi-criteria analysis, network analysis, and temporospatial analysis using geographic information systems are more readily available. Of course, such tools require the assistance of professionals in epidemiology and biostatistics. However, the growing availability of technical tools, such as EpiInfo from the Center for Disease Control and Prevention, make it possible for practitioners to apply directly epidemiologic principles in investigating disease and production issues. This presentation will highlight what can be done by practitioners while also presenting novel approaches that could provide additional support.

Application of epidemiological tools in investigating disease from voluntary disease databases

A. G. Arruda[2]

[a]Assistant Professor, Department of Preventive Veterinary Medicine, The Ohio State University, Columbus (Ohio), 43210, USA

In the absence of regulatory frameworks for diseases of economic importance in swine, voluntary disease monitoring and control programs have become common and important to improve health and productivity; and decrease disease spread. The collection of data over time and space allows not only for immediate action towards disease mitigation; but also for the application of a variety of analytic epidemiological tools in order to provide long-term value to swine veterinarians and producers; which in return facilitates and encourages participation and aids with program maintenance and sustainability. The objective of this presentation is to illustrate the application of diverse epidemiological tools to large volunteer-based databases for the improvement of disease understanding and consequently control and prevention. The porcine reproductive and respiratory syndrome (PRRS) is the example of choice for such applications since it is arguably the most economically devastating disease for swine producers, it is not a federally regulated disease; and there are considerable knowledge gaps in its epidemiology. During the past years, PRRS monitoring and control projects allowed for the development and application of tools involving spatial analysis, network analysis, infectious disease modelling, time series analysis, besides others; in order to better understand the occurrence, spread, and control of this disease under field conditions. A few highly applicable findings from research using Ontario's (Canada) and U.S.'s PRRS databases

include: i) even though airborne area spread of PRRS is anecdotally reported as the main route of spread between farms, spatial and network analysis revealed that service provider networks may play a greater role in defining PRRS status; ii) the presence of shrubs and trees; and farms located in areas with higher slopes were associated with fewer PRRS outbreak rates; and iii) PRRS's seasonality pattern varies according to geographical region in the U.S.

Epidemiologic Tools Used in Investigating the 2015 HPAI Outbreak

Brian McCluskey

USDA-APHIS VS

During the highly pathogenic avian influenza outbreak (HPAI) in 2014-2015, the USDA's Animal and Plant Health Inspection Service (APHIS) in partnership with poultry producers conducted epidemiologic investigations with the primary goal of rapidly providing mitigation and prevention information to producers and poultry companies. These investigations included: field-based observational studies with data collected through surveys and site visits; geospatial analyses; and on-farm sampling efforts. A case series investigation of turkey flocks and a case-control study of layer operations identified risk factors associated with the introduction of HPAI onto operations. Some of the statistically significant factors for mitigation were associated with straightforward improvements in biosecurity. Geospatial analyses focused on the role of wind speed and direction in the transmission of HPAI and although different analyses resulted in varying results the most stable predictor of increase odds of disease was the cumulative exposure to plumes over a 6-11 day period. APHIS supported on-farm air sampling efforts with the University of Minnesota's College of Veterinary Medicine. Air samples from both inside and outside affected poultry houses were determined to be positive for HPAI by RT-PCR. Low levels of genetic material were detected at distances of approximately 70-1000 meters. APHIS also conducted on-farm sampling of synanthropic wildlife at affected and unaffected layer operations to determine their role in the transmission of HPAI. HPAI virus was detected in two peri-domestic avian species. In all studies, rapid dissemination of results was emphasized to assist both regulatory agencies and producers in initiating prevention strategies.

Geospatial analysis and other epidemiological tools in understanding common poultry diseases

Louise Dufour-Zavala

Georgia Poultry Laboratory Network

With the goal of improving contagious disease control, over the past decade, several tools have been used to better understand the epidemiology of common poultry diseases in Georgia. Although industry decisions had been made with pathogen knowledge in mind, the addition of analysis of disease data greatly contributed to our disease control decisions working best in our environment. Examples of what will be presented includes: geospatial analysis of risk factors for LT and MS, climatic factors involved in LT outbreaks, simple statistical methods for the analysis of farm factors contributing to MS entry, as well as the use of epidemiological surveys to better understand the possible reasons for each Mycoplasma case using the biosecurity principles as a backbone will be discussed.

Epidemiological application to molecular techniques in understanding the introduction, spread and containment of poultry disease

Mark W. Jackwood

Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens GA 30602

Molecular techniques have increased our ability to analyze a large number of samples very rapidly. The techniques are also extremely sensitive, which has allowed diagnostic laboratories to move away from testing on isolated agents to directly analyzing clinical samples. This volume, speed and sensitivity of analysis allows for tracking the spread of

pathogens in the field like never before. In addition, molecular tests can provide highly specific gene sequence information that not only identifies the disease agent but also can be used to define the specific type/species/strain of the pathogen. That data has been used to characterize pathotype, identify vaccine strains and it can provide information on the genetic changes or evolutionary trajectory of the pathogen as it spreads in the field. As an example, molecular tools for the detection, identification and typing of infectious bronchitis virus will be discussed with special attention to analysis of the data to better understand the epidemiological nature of the virus. Using a molecular epidemiological approach, the genetic relationship, diversity and distribution of IBV strains circulating in the field can be elucidated. This is important for our understanding of the evolutionary changes in IBV associated with the emergence of new types and the affect vaccines play on those changes.

Enteric Health

Investigations of influencing factors on the gut health and microflora composition in layers

S. Rautenschlein[a], H. K. Scholtes[a], J. Kamphues[b], I. Rychlik[c], F. J. Hoerr[d]

[a]Clinic for Poultry, University of Veterinary Medicine Hannover, Hannover, Germany. [b]Institute for Animal Nutrition, University of Veterinary Medicine Hannover, Hannover. Germany. [c]Veterinary Research Institute, Brno, Czech Republic. [d]Veterinary Diagnostic Pathology, Fort Valley, Virginia, USA.

Animal health is influenced by the gut microflora, which is affected by feed, genotype, immune responses as well as management and environment. Our objective was to determine if differences in diet composition may modify gut health and microflora composition in commercial layers. On four farms layers either fed with a conventional or a fiber-enriched diet were randomly selected and sacrificed at the age of 24 – 26 and 55 - 56 weeks. Content of different gut sections was collected and investigated by Illumina sequencing. Body, liver and gizzard weight, as well as lesion development were determined. The body weight was comparable between feeding groups within one farm but different between farms ($P < 0.05$). No lesions were detected in necropsied birds. Only minor variations were observed in villi high, mucosal thickness and crypt depth of gut sections. The bird's age as well as the farm had the most influence on the microflora composition ($P < 0.05$), while the diet influence was less clear. Although the diet had a modifying effect on the microflora composition on each farm, differences varied between farms, suggesting a dominating effect of the farm environment itself.

The effects of direct-fed microbial supplementation, as an alternative to antibiotics, on growth performance, intestinal immunity, and epithelial barrier integrity in broiler chickens infected with *Eimeria maxima*

Inkyung Park[1], Youngsub Lee[1], Doyun Goo[1], E. Davis[2], N. Zimmerman[2], T. Rehberger2 and Hyun Lillehoj[1*].

[1]Animal Bioscience and Biotechnology Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, USDA, MD 20705;[2]Agro Biosciences Inc., 10437 Innovation Drive, Wauwatosa, WI 53226, USA

The objective of this study was to investigate the effect of dietary *Bacillus subtilis* supplementation on growth performance, intestinal integrity and cytokine, and tight junction protein of broiler chickens infected with *Eimeria maxima*. Fourteen-day-old broiler chickens (n=196) were randomly assigned to one of seven dietary treatments: two basal diets (CON and NC); CON+virginiamycin (AB1); CON+bacitracin methylene disalicylate (AB2); CON+*B. subtilis* strain 1781 (PB1); CON+*B. subtilis* strain 747 (PB2); CON+*B. subtilis* strain 1781+strain 747 (PB3). At day 7, all birds except CON were orally inoculated with *E. maxima*. Body weight and feed intake were measured at day 7 and 13. At day 10 and 13, jejunal tissue was collected from 6 randomly selected birds from each treatment group and jejunal lesion scores were recorded. At day 7, body weight gains of chickens fed PB2 and PB3 were increased (P=0.032) as much as AB2 group. At day

13, body weight gain and feed efficiency of PB2 group were increased (P<0.001) and had (P=0.005) the lowest lesion score among the treatment groups. Consequently, *B. subtilis* strain 747 supplementation improved growth performance and intestinal integrity of broiler chickens infected with *Eimeria* maxima.

Mechanism of action of antibiotic growth promoters: alteration of immune response and intestinal metabolome

Hyun S. Lillehoj

Animal Biosciences and Biotechnology Laboratory, ARS, USDA, Beltsville, MD 20705, USA.

Antibiotic overuse and abuse on a global scale has led to the emergence of multi-drug resistant "superbugs" from food animals and humans. The United States Food and Drug Administration has requested that agriculture producers discontinue sub-therapeutic dosing of antibiotics into animal feed, which for over 60 years, was the common practice to promote their economic value by increasing feed efficiency and growth. To develop non-antibiotic feed additive(s) that promote the growth and health of commercial poultry, we initiated intercorrelated omics-based strategy to better understanding of mode of action of antibiotic growth promoters (AGP) and dietary modulation of immunity that will ultimately improve on-farm animal performance. It is believed that over 100 trillion microbes make up the animal gut microbiome. These microorganisms decompose indigestible substances such as fiber, providing an energy source for their host. They also metabolize ingested food to produce various beneficial "postbiotic" compounds, including amino acids, vitamins, and short-chain fatty acids such as acetic acid and butyric acid that impact appetite, growth and immunity. This talk will primarily focus on our recent study to identify metabolites produced in response to growth promoting antibiotics as post-transcriptional markers that correspond to increased feeding and growth for better understanding of the mechanisms of action involved in antibiotic growth promotion. These results provide the framework for future studies to identify natural chemical compounds to improve poultry growth performance without the use of in-feed antibiotics.

Efficacy of supplemented *Bacillus subtilis* DSM 17299 in broiler chickens fed standard and energy, protein- and amino acid- reduced diets

M. Lang[1], J. Schleifer[1], R. Koedjik[1], A.B. Kehlet[1], J. Zentek[2], F. Goodarzi[2] and K. Männer[2]

[1]Chr. Hansen A/S, Boege Allé 10-12-2970 Hoersholm, Denmark [2]Freie Universität Berlin, Königin-Luise-Straße 49, 14195 Berlin, Germany

Bacillus subtilis DSM 17299 (BS) can produce a wide range of digestive enzymes potentially advantageous to digest feed in the gut of broiler chickens. The objective of this study was to evaluate the effect of addition of a BS in standard and energy - and protein/amino acid- reduced diets fed to broilers from d 1 to d 42 of age. A total of 720-day-old Cobb male chicks were distributed in 48 floor pens (15 birds/pen), allocated to 6 dietary treatments (8 rep/treat). Body weight (BW), feed intake, and mortality adjusted FCR were assessed at each feed change. The results were analyzed by one-way ANOVA. The addition of BS improved BW by 13.5 g in the standard diet. In reduced T2 the addition of BS improved BW by 48.4 g and in T3 by 57.8 g. FCR was slightly (standard diet) or significantly (reduced diets) reduced by 1.8% or 3.1 and 3.0% respectively compared to the corresponding diets. Other parameters were improved comparing to other treatments respectively and will be described. The current study shows that BS can be used effectively to improve broiler performance and to overcome the performance loss associated with a reduction in nutrient density.

Avian Influenza

Development of a novel avian influenza live virus vaccine based on disruption of M2/M42 gene expression

Darrell R. Kapczynski[1], Klaudia Chrzastek[1], Karen Segovia[1], Lonneke Vervelde[2], Lita Murphy[2], Mary Pantin-Jackwood[1], David L. Suarez[1] and Paul Digard[2]

[1]Exotic and Emerging Avian Viral Diseases Research Unit, U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, 934 College Station Road, Athens, GA, 30605; The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, Scotland, U.K. EH25 9RG

Influenza A viruses are genetically diverse pathogens that can infect various hosts including birds, swine, and humans. Vaccines and vaccination have emerged during the past three decades as essential tools in influenza control. Their use in poultry can increase resistance to infection, prevent illness and death, reduce virus replication and shed, and reduce virus transmission to susceptible birds. Historically, only killed influenza virus vaccines are used in animals due to the risk of reversion of the virus in the vaccine to an enhanced virulent state or recombination of the virus in the vaccine with other influenza viruses. Recently, recombinant influenza virus vaccines have been licensed for use in poultry. These vaccines express a single key influenza immunogen, the hemagglutinin (HA) protein. Killed (inactivated) influenza virus vaccines only induce systemic antibodies against the virus, whereas a live replicating vaccine virus induces antibodies, mucosal and cellular immunity. Currently available recombinant vaccines only induce immunity against the HA protein. The immunity produced by live virus vaccines is generally more effective as it recognizes all viral proteins, and is more cross-reactive than the immunity induced by inactivated vaccines. In these studies we generated live virus low pathogenic avian influenza H5N2 vaccines based on disruption of segment 7 expressing either M2 or M42 protein. The vaccines replicated in chickens and induced a protective immune response against homologous and heterologous highly pathogenic avian influenza challenge. The mutations resulted virions with increased size and differential morphology compared to wild-type virus. Importantly, when applied to chickens the vaccine virus did not transmit to susceptible cohorts demonstrating a decreased ability to spread. Taken together, these studies demonstrate the potential of live avian influenza virus vaccines with increased protection and decreased transmission potential.

Broader protection from a recombinant live vectored vaccine than inactivated avian influenza vaccines in chickens against diverse H5 highly pathogenic avian influenza viruses

Miria Ferreira Criadoa, Dong-Hun Leeab, Kateri Bertranac, Jung-hoon Kwona, Lindsay Killmastera, David E. Swayne[a]

[a]Southeast Poultry Research Laboratory, USDA-ARS, 934 College Station Rd., Athens, GA 30605, USA.

Control of H5Nx highly pathogenic avian influenza A viruses has been difficult using stamping-out programs alone. Thus necessitating the use of diverse vaccine technologies in some countries as an additional control tool. However, empirical observation suggests a difference in the protection by different vaccine technologies against antigenically diverse field viruses. In this study, three-week-old SPF leghorn chickens received a single dose of either an inactivated vaccine (rgH5N1 contained the HA gene from clade 2.3.4.4 A/Gyrfalcon/Washington/40188-6/2014 (H5N8) HPAIV in the backbone of A/Puerto Rico/8/1934 [H1N1] vaccine strain) or rHVT-H5 vaccine [same HA insert above]. Three- weeks post-vaccination birds were challenged with five different H5 HPAI viruses to evaluate protection. All five challenge groups vaccinated with the rHVT-H5 had ≥70% survival. In contrast, the inactivated vaccine provided ≥80% protection against four challenge viruses while one challenge virus achieved only 20% protection. Analyzes showed higher protection when the amino acids in the antigenic sites of HA between vaccine and challenge viruses are closely related. Independent of the vaccine and challenge virus used the OP and CL shedding was significantly reduced

compared to the sham group at 2 dpc ($p < 0.05$). Virus shedding differences between vaccines were only observed at 4dpc swabs from birds challenge with H5N1, clade 2.1.3 and clade 2.3.2.1 group B viruses. The presence of pre-challenge antibodies titers was associated with protection. Therefore, our study demonstrated the live vectored vaccine provided broader protection and may require less frequent updates to HA insert than the inactivated vaccines as field viruses antigenically diverge.

Improvement of an NS1-truncated live attenuated influenza vaccine by selection of viral subpopulations with enhanced interferon-inducing capability

Amir Ghorbani[1,2], John M. Ngunjiri[1], Michael C. Abundo[1,2], Mohamed Elaish[1], Hana Ji[1], Kara Taylor[1], Chang-Won Lee[1,2,*]

[1]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, United States of America
[2]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, United States of America

The continued occurrence of highly pathogenic avian influenza outbreaks in poultry has necessitated the development of preventative measures beyond biosecurity programs. Additionally, the currently available poultry vaccines have poor cross-reactivity against the emerging field strains with new antigenic makeups which warrants the development of more broadly reactive vaccines. We have developed an NS1-truncated live attenuated influenza vaccine (pc4-LAIV) that provides partial but broad cross-protection against shedding of heterologous low and high pathogenicity avian influenza viruses in chickens. The efficacy of pc4-LAIV correlates with its ability to generate and maintain large subpopulations of interferon-inducing and defective-interfering viruses *in vitro*. This study was conducted to further improve pc4-LAIV by deconstructing its viral population through plaque purification and selecting new vaccine candidates based on the interferon-inducing capacity as an *in vitro* phenotypic marker. From one-hundred plaque isolates, we were able to identify several candidates that induce significantly higher levels of interferon in chicken embryonic cells compared to the original virus. These candidates also produce high levels of defective viral genes, especially within their polymerase genes. Further analyses are underway to gain more insight into the genomic characteristics of high and low interferon-inducing subpopulations through high throughput next generation sequencing. Profiles of single nucleotide polymorphisms and deletion junctions of defective viral genes of the best vaccine candidates will be utilized to design a live vaccine backbone that is more efficacious than our original pc4-LAIV.

Importance of Vaccine seed strain matching for the control of H9N2 avian influenza

David L. Suareza and Liz Puscha

[a]Southeast Poultry Research Laboratory, 934 College station Rd, Athens, GA 30605

The H9N2 avian influenza virus subtype is commonly found in poultry around the world and at least four unique poultry adapted lineages have been described including the Korean, European, Y280, and G1. The H9N2 subtype in SPF birds in the laboratory typically causes no mortality and is considered to be low pathogenic. However, in the field in the presence of co-infections and poor environmental conditions economically important clinical disease with some mortality is commonly observed and H9N2 is an important respiratory pathogen. These poultry adapted viruses are highly infectious and transmit easily. Vaccination has become an important control tool to reduce the impact of the virus. Unfortunately, H9N2 viruses are genetically and antigenically diverse, and vaccines may not always protect from infection. Experimental studies were performed with antigenic variants of the G1 virus lineage to examine protection with killed adjuvanted vaccines with both homologous and heterologous challenge strains. The G1 lineage is probably the most widespread of the virus lineages occurring in Southeast Asia, the Middle East, and in many countries in Africa.

Because clinical disease and mortality are uncommon in SPF chickens, emphasis on virus shedding at 2, 4 and 7 days after challenge will be used. Additional studies looking at transmission of the virus in vaccinated birds will be compared with homologous and heterologous vaccination. The goal of the study is to try to understand how closely matched vaccines need to be the field strains to consistently get reliable protection to allow an understanding when seed strains need to be updated.

Development and evaluation of live attenuated vaccines against highly pathogenic H7N3 avian influenza virus

Stivalis Cardenas-Garcia, Silvia Carnaccini, Ginger Geiger, Lucas Ferreri, Daniela Rajao, and Daniel Perez

Poultry Diagnostic and Research Center, Department of Population Health, The University of Georgia, Athens, GA, USA

Episodes involving highly pathogenic avian influenza (HPAI) H7N3 viruses have been reported in Mexico stating in 2012. Since then, Mexico has taken intensive measures on the attempt to control the outbreaks occurring in different regions of the country, devastating the poultry industry. Current vaccines against H7N3 viruses have been shown to be poorly immunogenic and unable to confer adequate protection, underscoring the need for the development new of AI vaccines able to mount a more robust immune response and provide better protection. Generally speaking, live attenuated vaccines are able to induce stronger cellular immune responses compared to inactivated vaccines, thus reducing viral replication and shedding, contributing to decreased transmission rates. In the past, we have developed strategies to generate safe live attenuated influenza vaccines (LAIV). Our strategy for genome re-arrangement has been proven to allow for the generation of potentially good LAIV candidates. In the present study, two rearranged H7N3 LAIV viruses have been developed by reverse genetics. Growth kinetics were assessed in MDCK cells at different temperatures, demonstrating their impairment to replicate at high temperatures compared to the parental virus. Genome stability of the re-arranged viruses was evaluated by serial passages in MDCK cells, showing that the modified genes remained stable. To assess protection efficacy of these vaccine candidates, one-day-old SPF White Leghorn chickens were vaccinated and boosted with the either one or both viruses, and then challenged with a Mexican HPAI H7N3 virus. Antibody response, protection against clinical disease, mortality, and viral shedding upon challenge have been evaluated.

Avian Influenza H6 Virus-like Particles Confers Potent Immunogenicity and Protective Efficacy in Chickens

Wan-Zhen ZHUa, Yi-Chi WENa, Ting-Chih CHENa, Shu-Yi LINa, Hui-Wen CHEN[a,b]

[a]Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
[b]Research Center for Nanotechnology and Infectious Diseases, Taipei, Taiwan

H6 avian influenza viruses (AIVs) have circulated in domestic chickens in the field of Taiwan and the world for decades since its first identification from turkeys in Massachusetts, USA in 1965. H6 AIVs were identified as potential progenitors of the highly pathogenic H5N1 AIV that emerged in Hong Kong in 1997 and H5N2 AIV in Taiwan in 2004, and these H6 viruses continue to circulate among the bird populations in Asia. The unique human and canine infection cases of H6N1 virus were reported highly homologous to chicken isolates in Taiwan. Towards containing the disease, there is a pressing need to develop a safe and effective vaccine for an influenza H6 pandemic preparedness. This study aims to prepare an immunogenic virus-like particle (VLP) that consists of hemagglutinin (HA) and matrix protein 1 (M1) derived from the chicken isolate for vaccine development. Full length HA and M1 protein genes were cloned and expressed using a baculoviral expression system, and the VLPs were generated by infecting insect cells with the recombinant baculovirus. VLPs were purified from the supernatant of the insect cell cultures. VLPs' size, morphology, antigenicity, and hemagglutination activity resembles the native

virions, as examined by the nanoparticle tracking analysis and transmission electron microscopy. In animal experiments, SPF chickens receiving the H6 VLP in combination with ISA71 or CpG-encapsulating nanoparticle adjuvant showed superior and long-lasting H6 virus-specific serum IgG and hemagglutination inhibition antibody titer, which last at least 112 days. Following H6N1 viral challenge, the vaccinated chickens showed significant reduced viral cloacal shedding and the viral load in the lung and kidney. In addition, the VLP generate prominent cross-reactivity against the human H6 isolate. In summary, the H6 VLPs confer desirable immunogenicity and protection and may serve as a candidate for vaccine development against influenza A/H6 virus infection.

Mutations in PB1, NP, HA and NA Genes Contribute to Altered Pathogenicity and Viral Shedding of H5N2 Highly Pathogenic Avian Influenza Virus in Chickens and Mallards

Sungsu Youk[a], Christina Leyson[a], Diane Smith[a] and Mary J. Pantin-Jackwood[a]

[a]*Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA, USA*

H5N2 highly pathogenic avian influenza (HPAI) emerged in the United States at the end of 2014 and caused severe poultry outbreaks during 2015. In previous infectivity and pathogenicity studies the poultry H5N2 isoates had increased adaptation to chickens and decreased virus shedding in mallards, when compared to the wild duck index H5N2 virus. To establish the molecular basis of this phenotype, we generated H5N2 recombinant viruses using the index H5N2 virus (A/Northern pintail/Washington/40964/2014) and a late chicken isolate(A/chicken/Iowa/13388/2015). Single or multiple gene reassortants were used to determine which viral genes contribute the differing pathogenicity in chickens and mallards by means of replacing the index H5N2 genes with the chicken isolate genes. The viruses bearing the HA or NA gene of the chicken isolate resulted in decreased viral shedding in mallards, and viruses with exchanged PB1 and NP genes showed different tissue tropism. Infection with the NP reassortant virus caused very early mortality in chickens. Notably, the HA reassortant virus was able to transmit to contact chickens. To further identify the role of the mutations between the viruses, *in vitro* studies including viral growth kinetics in chicken and duck origin cells were conducted. These results indicate that mutations in the PB1, NP, HA and NA genes that occurred during the H5N2 HPAI virus circulation in poultry are associated with viral adaptation to chicken.

Overlapping genes of the avian influenza virus M segment: Role of M2/M42 in evolution of highly pathogenic avian influenza virus

Karen Segovia[a], Klaudia Chrzastek[a], Lita Murphy[b], Lonneke Vervelde[b] Paul Digard[b] and Darrell R. Kapczynski[a]

[a]*Exotic & Emerging Avian Viral Diseases, SEPRL, ARS, USDA, 934 College Station Rd, Athens, Ga 30605;* [b]*The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian EH25 9RG.*

Poultry products represent an important source of affordable animal protein throughout the world. However, poultry production is still vulnerable to a range of infectious diseases. Foremost among these is avian influenza (AI), which can result in high mortality and significant economic losses. Although the principal virulence determinant of AI is known, other uncharacterized genetic determinants contribute to the pathogenesis. The mechanisms by which these changes modulate and contribute to viral pathogenesis and transmission are incompletely understood. Segment 7 of influenza A virus (IAV) produces up to four messenger RNAs (mRNA), including unspliced transcripts for M1, and spliced mRNA2 for the M2 ion channel. The M2 ion channel of plays an essential role during both viral entry and egress. We have previously described a rare variant of the M2 channel, M42, with an altered ectodomain that can functionally replace M2 in the viral lifecycle. In this study, we generated three reverse genetics viruses expressing either or both M2 and M42 proteins. We characterized differences

in morphology and replication kinetics between viral strains by immunofluorescence, electron microscopy, and inoculation in different culture substrates. M2 and M42 display notably different intracellular localization, with the former, predominantly localized in the plasma membrane and the latter to the Golgi apparatus. Taken together, our results describe a new functional motif in the M2 ectodomain that helps explain the functional constraints that underlay its conservation and may play a role in protecting the hemagglutinin during its initial progression from low to highly pathogenic forms.

The Molecular Determinants of Antibody Recognition and Antigenic Variations of the HA of H9N2 Viruses.

Silvia Carnaccini[a], Nicola S. Lewis[b], Lucas M. Ferreri[a], Jefferson Santos[a], Adebimpe Obadan[a], Stivalis C. Garcia[a], Eugene Skepner[b], David Burke[b], Ian Brown[c], Daniela Rajao[a], Daniel R. Perez[a].

[a]Population Health Department, University of Georgia, School of Veterinary Medicine, Athens, Georgia, USA [b]Centre for Pathogen Evolution and WHO Collaborating Centre for Modelling and Control of Emerging Infectious Diseases, Department of Zoology, University of Cambridge, Cambridge, UK cAnimal and Plant Health Agency (APHA), Department for Environment, Food & Rural Affairs, Weybridge, UK

Avian influenza viruses of the H9N2 subtype are an important cause of disease and economic losses in poultry worldwide. They are endemic in poultry in Asia and pose a threat to global human health as zoonotic agents (human infections have been reported across Asia, Bangladesh and Egypt), and as gene donors to other zoonotic avian influenza viruses such as the 1997 Hong Kong H5N1 outbreak, and the recent Chinese H6N1, H7N9 and H10N8 outbreaks. Little is known about these viruses' antigenic profiles and, although phylogenetic analysis provides important information for viral diversity, it alone cannot clearly predict levels of cross-reactivity between isolates within the same subtype. In the present study, phylogenetics, reverse genetics and antigenic cartography are applied to provide a deeper understanding of the antigenic variations between H9 clades and how they correlate to immunogenicity. Ten different consensus HA1 sequences were constructed from phylogenetic analysis of the global H9 sequences and ten viruses were generated by reverse genetics over four different backbones. Most of the reverse genetics viruses were able to rescue on the four different backbones. Subsequently, the viruses were used to generate antisera in quails and ferrets to be tested by hemagglutination inhibition assay and analyzed by antigenic cartography. The antigenic/antibody relationship between the consensus viruses showed comparable specificities between animal species. As expected, phylogenetic analysis did not correlate with the antigenic cartography, but it aided in the initial clustering of strains. A refined antigenic map and amino acids involved in modulating H9 HA antigenic activity will be presented.

Determining molecular markers of avian influenza virus adaptation in poultry by full genome sequencing of different lineage viruses

Mary J. Pantin-Jackwood[a], Sungsu Youk[a], Dong-Hun Lee[b], Christina Leyson[a], Diane Smith[a], and David E. Swayne[a]

[a]Southeast Poultry Research Laboratory, National Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Athens, GA. [b]Department of Pathobiology and Veterinary Science, The University of Connecticut, Storrs, CT.

We have shown that the pathobiology of avian influenza virus (AIV) changes as it adapts to different avian species. Virus adaptation is demonstrated by increased virus infectivity, increased replication titers and duration of virus shed, and improved transmission. To understand how AIV's change as they passage in wild avian reservoir species and domestic poultry, and to identify molecular markers for AIV adaptation in poultry, we examined whole genome sequences of viruses from current and ongoing AIV outbreaks and from chickens and mallards experimentally infected with different AIVs. Sequences from different AIV lineages

including, H5NX highly pathogenic (HP) AIV (clade 2.3.4.4), Mexican H7N3 HPAIV and H5N2 low pathogenicity (LP) AIV, and U.S H7N8 HPAIV, were analyzed. Viral changes related to increased adaptation in poultry were identified in most viral genes but were more common in the polymerase complex genes. Many of these changes have been also reported in other studies to be associated to increased virulence and adaptation in chickens, indicating that specific virus mutations might be markers of poultry adaptation. This information is important because viruses that are well adapted to poultry are highly infectious and transmissible, contributing to faster spread between premises.

In vivo and *in vitro* differences between two H5N8 clade 2.3.4.4 highly pathogenic avian influenza viruses clade 2.3.4.4

Christina Leyson, Sungsu Youk, Diane Smith, and Mary Pantin-Jackwood

Southeast Poultry Research Laboratory, National Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture

Highly pathogenic avian influenza (HPAI) viruses subtype H5N8, clade 2.3.4.4, have been causing outbreaks in wild birds and poultry since it was first described in 2013. Experimental infections with two H5N8 viruses belonging to distinct groups within clade 2.3.4.4 showed significant differences in virulence in mallards, with no mortality in mallards infected with a North American isolate from 2014, and 80% mortality in mallards infected with a European virus from 2016. The two viruses were not well adapted to chickens, with similar high doses of the viruses needed to infect the birds. In *in vitro* studies, the two viruses replicated differently in chicken and duck origin cell cultures. To determine if the hemagglutinin (HA) or polymerase complex genes are implicated in increased virulence in mallards, we also performed assays that test the functionality of proteins encoded by these genes. To test binding characteristics of the HA, recombinant HA proteins from the two viruses were expressed, purified, and subsequently used as a reagent for histochemistry on host tissues. The extent of protein binding onto host tissues was quantified and compared. To test for the functionality of polymerase complex, the PB2, PB1, PA, and NP genes were cloned into an expression vector and co-transfected with reporter plasmids into HEK 293 cells to determine polymerase activity. The data generated from these studies help explain the differences observed in H5N8 HPAI virus infectivity and replication in different bird species.

Alterations in H5 Highly Pathogenic and Low Pathogenic Recombinant Hemagglutinin Tissue Tropism associated with Increasing Age in Selected *Anseriformes* and *Galliformes* Bird Species

Carmen Jerry[a,b,] Christina Leyson[d], David Stallknecht[c], Monique S. França[a,b]

[a.]Poultry Diagnostic and Research Center, 953 College Station Road, Athens, Georgia, 30602. [b.] Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, 30602, USA. [c.]Southeastern Cooperative Wildlife Disease Study, 589 D.W. Brooks Drive, Athens, Georgia, 30602. [d.]Southeast Poultry Research Laboratory. 934 College Station Road, Athens, Georgia 30605

Intercontinental spread of Influenza A viruses (IAV) has been linked to introduction of novel H5 viruses to the United States, posing severe risks to poultry species. Outbreaks of highly pathogenic avian influenza (HPAIV) often causes infection and death in older birds (laying chickens and turkeys) compared to younger birds (broiler chicken). Conversely, experimental trials have demonstrated that younger birds are more susceptible to clinical disease caused by HPAIV, compared to older counterparts. To evaluate differences in H5 HA protein binding and tissue tropism, recombinant HA proteins were generated using gene sequences from low pathogenic (LPAIV) and HPAIV H5 IAVs. Broiler chickens, egg layers, turkeys, Mallard, Pekin, Muscovy, and Muscovy mule ducks were selected at the age groups: 1-2-weeks, 5-6-weeks, and 8-weeks-old. Samples of respiratory, and intestinal tract, cloacal bursa, kidney, brain and liver were selected for HA protein binding, using protein histochemistry. Among *Anseriformes*, 5-week and 8-week old birds had stronger HPAIV HA binding to the

trachea, lung and cloacal bursa compared to younger birds. Significant differences were not observed in other tissues. The LPAIV HA had no significant differences in binding across age groups. Among *Galliformes*, stronger HPAIV HA binding was observed in the trachea and bronchi of 1-day, and 6-week-old pullets, with no alterations in HA binding in other tissues. Turkeys had no significant differences among age groups with both HAs. This study is expected to provide insight into age-related differences in tissue tropism of IAV which may help explain host-related differences in susceptibility to infection during outbreaks.

Challenges in Post-VE Environmental Sampling and the Use of the VetMAX Gold AI Detection Kit

Len Chappell

Georgia Poultry Laboratory Network

NVSL provides a standard protocol for the collection of environmental samples after the premises have been thoroughly cleaned and disinfected following AIV exposure. Because chicken house construction and design do vary to some degree across the Industry, it is important for the testing team to develop a sampling plan specific to the facility being tested. Our post Virus Elimination collection team took samples in clean and vacant houses from twelve locations per house from four different companies in non-AIV event facilities. A few of the environmental samples for each of the companies' houses were spiked with an equivalent concentration of AIV antigen, and all samples were analyzed using the VetMAX Gold AIV Detection kit. Sampling challenges, deviations from NVSL protocols, as well as PCR results are discussed in detail.

Persistence of LPAI in carcass composting and bedding litter treated with acidifier amendment

Alejandra Figueroa[a], Ali Abdelnabi[b], Sagor Biswas[a], Pramod Pandey[a], Rodrigo Gallardo[a]

[a]UC Davis School of Veterinary Medicine, Davis, CA.
[b]Vanderbilt University, Nashville, Tennessee.

Avian influenza (AI) viruses have had a major impact on the poultry industry worldwide. Research is needed to understand its interaction within commercial poultry barns. Our current study focuses on the effect of an acidifier litter amendment, commonly used for ammonia control, on the persistence of LPAI in broiler and turkey litter. Broiler and turkey litter, with or without amendment, were spiked with LPAI. Samples were collected before spiking and at several timepoints after. In addition, we investigated carcass composting as an effective method to inactivate LPAI. Results of these two experiments will be discussed.

Epidemiology

Epidemiological approaches to investigate the determinants of poultry diseases and productivity

Robert Wills

College of Veterinary Medicine, Mississippi State University

The poultry industry has a well-deserved reputation for its ability to collect production and disease data. However, fully leveraging that data to make informed decisions in poultry production and disease control is challenging. The focus of this presentation is how to utilize epidemiological approaches to use routinely collected data as well as data from well-designed observational studies to better understand poultry diseases and identify effective interventions. Epidemiological approaches to disease investigation focus on describing and quantifying the associations between disease outcomes or loss of production and agent, host, and

environmental determinants or risk factors. Observational study designs, such as case-control, cohort, and cross-sectional studies, are commonly used to study disease as it naturally occurs rather than in the research laboratory. Although these study designs allow research to be conducted in the field under real world conditions, they are also prone to bias. Understanding the strengths and limitations of these approaches enables their use to better understand the etiology and dynamics of disease. Fundamental principles that are essential to effective study design, such as determining the experimental unit, sample size, sampling strategies, and controlling bias, will be highlighted. In addition to the proper design of these studies, proper statistical analysis of the data is also crucial to assessing the associations between the risk factors and disease occurrence. The presentation will give an overview of some of the statistical methods frequently used to discern the relationships between host, pathogen, environment, and management practices and the occurrence of disease.

Understanding how multiple production variables affect performance using statistical freeware

Dave Fernandez

Select Genetics, 1800 Technology Dr. NE, Willmar, MN 56201

The study evaluates production data from a 24-month period and analyzes how specific production variables affect performance in the face of varying types of production systems and disease exposures. An investigator looking at retrospective production data and assessing the role of multiple variables (breed, season, production interventions such as the use of litter amendments or probiotics and other related variables) on production performance has to use statistical models. Aside from understanding the relationship of production variables to performance, these same models allow the user to evaluate how individual variables interact with each other. Since most production companies either do not invest in commercial statistical packages or divert analytical research to academic institutions, readily available statistical freeware may encourage these companies to evaluate their own data to improve efficiency.

Application of Remote Sensing Technologies to the Epidemiology of Avian Influenza and Other Poultry Diseases

Todd Kelman[a], Jeff Buler[b], Elliott Matchett[c], Maurice Pitesky[a]

[a]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, 1089 Veterinary Medicine Dr. VM3B, Davis CA, 95616.
[b]Department of Entomology and Wildlife Ecology, University of Delaware, 246 Townsend Hall, Newark DE, 19716.
cUSGS-Western Ecological Research Center 800 Business Park Drive, Suite D, Dixon CA, 95620.

Surveillance efforts for avian influenza viruses (AIv) within wild waterfowl in the United States have dropped precipitously in the past year due to budget shortfalls, underscoring the importance of utilizing advanced technology to focus the limited resources available for infectious disease monitoring. Additionally, traditional surveillance methods neglect the temporal variability of waterfowl habitat, including natural and man-made wetlands, dynamic agricultural land management and the effect of climate change on these landscapes. We are employing a multimodal spatio-temporal approach to track waterfowl and their habitat, utilizing a combination of NEXt generation weather RADar (NEXRAD), satellite data (MODIS and Landsat), and machine learning land-cover classification (NASS). This utilization of radar and satellite imagery to dynamically track waterfowl with the intent to quantify infectious disease risk is a novel use of remote sensing technology. NEXRAD, satellite, and NASS data are all free to access, and require various degrees of processing before they can be applied to habitat modeling and infectious disease surveillance efforts. We discuss various methods and tools available to access and analyze these data sources, including limitations and specific impediments that we have encountered in utilizing them for our own efforts. Resources for further

study of these methods and tools will be provided. The use of machine learning techniques including Classification and Regression Tree based models (e.g. Random Forest and Boosted Regression) and the use of the R programming language and the R Studio environment to analyze remote sensing data for prediction of infectious disease risk will also be discussed.

Tactical Epidemiology: Analysis Becomes Action During the Virulent Newcastle Disease Response in California

Annette Jones[a], Andrea Mikolon[b]

[a]Director and State Veterinarian, Animal Health and Food Safety Services, California Department of Food and Agriculture, Sacramento, California.
[b]Veterinarian Specialist, Animal Health Branch, California Department of Food and Agriculture, Sacramento, California.

Epidemiologists play increasingly diverse and critical roles during State and Federal emergency response to disease outbreaks. While traditional roles involving data trend, genome sequence, point source and risk factor analysis remain important, during the 2018 response to virulent Newcastle disease in California backyard poultry, "Tactical Epidemiologists" embedded in Operations were critical to sorting priority actions and influencing tactics. Virulent Newcastle disease is a foreign virus to North America and is considered a Select Agent by Homeland Security because of the damage it can do to our nation's economy if introduced into commercial poultry. Since May 2018, the California Department of Food and Agriculture and U.S. Department of Agriculture have worked together to eliminate this virus from backyard poultry in Southern California to prevent such a catastrophe. With more than 18 million people living in the greater Los Angeles area, a large percentage of whom own backyard poultry, using surveillance and diagnostic teams optimally is challenging. With limited resources, strategic use of field personnel becomes even more important. This challenge is not unique to the current semi-urban outbreak, but the number of premises and complex interactions in the at-risk population, make the value of the embedded epidemiologist particularly evident. Tactical Epidemiologists debrief field staff, conduct area reconnaissance, sort through field records, use several GIS and other mapping tools, and evaluate historical and non-traditional data sets to help Operations Section leaders refine tactics and prioritize field assignments. This bridging skill between traditional epidemiology and action on the ground will likely contribute to optimal use of resources during any emergency response to an outbreak and will be a part of the California response for the foreseeable future.

A retrospective study of variables associated with the incidence of necrotic enteritis in No Antibiotics Ever broiler complexes in the USA

Andrew Bishop

University of Georgia CVM

Due to the growth in popularity of No Antibiotics Ever (NAE) production within the US broiler industry in recent years, producers have found prevention of necrotic enteritis to be increasingly complex and frustrating. The vast number of possible predisposing and exacerbating factors form a complicated web of information, which may not be possible to interpret simply by looking at recent records or by on-farm evaluation. This study attempts to use an epidemiological approach to identify trends within complexes during the transition from conventional production to NAE. Records were obtained from several small-bird NAE complexes within the same company, both before and after switching to an NAE program, to determine changes or trends that occurred during the transition. Data from outside of the company will be considered as well, so as to identify any outside variables which may have an association with necrotic enteritis. Once trends are identified, they will be analyzed using current epidemiological tools, to determine if a significant association exists. The primary goal of this study is to demonstrate that available tools will allow producers to quantify factors closely associated with the ongoing issue of necrotic enteritis.

An Epidemiological Investigation of An Emerging IBD Variant in Broilers in BC, Canada

[A]Babak Sanei, [B]Stewart.J. Ritchie, [C]Neil Ambrose, [B]Gigi Lin, [D]Davor *Ojkic*

[A]Zoetis Canada Inc. Montreal, Quebec, Canada. [B]Canadian Poultry Consultants Limited, Abbotsford, BC, Canada, [C]Ambrose Poultry Consulting, Abbotsford, BC., Canada, [D]Univ. of Guelph, Guelph, Ontario, Canada.

In early fall of 2017, several integrators in Fraser valley, BC, Canada observed an unexpected rise in IBDV antibody titers (by ELISA) in blood samples from broiler flocks with slight increase in condemnation rates. During this epidemiological investigation, several farms from various companies were selected and over 3 consecutive placements, blood and bursal tissue samples were taken for serology, molecular diagnostics and genotyping. It was determined that the same identical variant was present in almost all affected farms. Based on genotyping results, the variant was very related to IBDV Del E strain with certain differences in its genetic makeup. Although initially it was thought shift in Del E has resulted in emerging of this variant, comparison with historical samples taken from same region in 2015 demonstrated that it is the same variant strain of IBDV. Several vaccine programs were also used to address this emerging IBD cases in this dense broiler production region of BC, Canada.

Wealth of Knowledge

2019 Research Priorities of the American Association of Avian Pathologists

Natalie Armour[a], Mark Burleson[b], Eric Gingerich[c], Seiche Genger[d], Travis Schaal[e], John Glisson[f], Naola Ferguson-Noel[g], and John Smith[h]

[a]Poultry Research and Diagnostic Laboratory, Mississippi State University
[b]Association of Veterinarians in Broiler Production
[c]Association of Veterinarians in Egg Production
[d]Association of Veterinarians in Turkey Production
[e]Association of Poultry Primary Breeder Veterinarians
[f]U.S. Poultry and Egg Association
[g]Poultry Diagnostic and Research Center, University of Georgia
[h]AAAP Board of Directors

The AAAP Research Priorities Committee (RPC) aims to serve the U.S. poultry industry by ensuring that the research priorities of the industry are identified and communicated to researchers and research funding agencies. Towards this end, the RPC conducts annual surveys of veterinarians in poultry production in the United States, represented by the memberships of the Association of Veterinarians in Broiler Production (AVBP), the Association of Veterinarians in Egg Production (AVEP), the Association of Veterinarians in Turkey Production (AVTP), and the Association of Poultry Primary Breeder Veterinarians (APPBV). For the 2019 survey year, an important goal of the RPC is to redesign and standardize the research priorities survey instruments for these associations. To achieve this goal, the AVBP, AVEP and AVTP presidents, together with one or two members of each association and with the input of the APPBV president, will generate complete lists of topics considered current research needs for their association in six categories: Health/Disease, Management/Environment, Animal Welfare, Food Safety, Diagnostic Tools, and Vaccines and Pharmaceuticals. The RPC will use these lists to design electronic surveys for veterinarians in broiler, egg and turkey production, incorporating a five-point scoring system for each

research need. Completed survey results will be analyzed and used to generate ranked lists of each association's top research priorities. The 2019 AAAP research priority lists will guide researchers and funding agencies to conduct and fund applied research that addresses the specific needs of the poultry industry.

USDA- National Poultry Improvement Plan Update

Elena Behnke

USDA- National Poultry Improvement Plan

The National Poultry Improvement Plan (NPIP) is a voluntary disease control program for the poultry industry in the United States. The objective of the NPIP is to provide a cooperative Industry-State-Federal program through which new diagnostic technology can be effectively applied to the improvement of poultry and poultry products throughout the country. All 50 states voluntarily participate in the NPIP and over 100 laboratories. During the 2018 NPIP Biennial Conference, over 325 members of the poultry industry voted on changes to the NPIP federal regulations. This presentation will provide an overview of the changes which have been approved by USDA and will become requirements for the US poultry industry.

Creating a New Cross Commodity Retail Labeled Animal Production Standard Based on the Principles of One Health

G. Donald Ritter

Mountaire Farms Inc., Millsboro DE

In the U.S. the way that antibiotics are used – or not used - during the raising of animals is becoming an integral part of the marketing strategy used to sell meat and poultry products by many producers. Unfortunately, most consumers are unfamiliar with animal agriculture and are increasingly confused by food labels related to antibiotics and animal production practices. The meanings and implications for consumers and animals of antibiotic related labels used by the food industry such as "No Antibiotics Ever" and "Responsible Antibiotic Use" will be discussed. There are unintended consequences and tradeoffs to these single attribute labels that are part of a package based labeling system. An alternative approach to food labels is a program or systems-based certification process that does not require diversion of product. A balanced multi-point standard that addresses several important areas in animal agriculture, including responsible antibiotic use that is respectful of animal welfare, may be a better solution to provide an affordable and sustainable labeled alternative for meat and poultry that will satisfy the needs of many consumers. A new cross commodity animal production certification program currently in development based on the principles of one health will be discussed.

Surviving the Hurricane of a Lifetime…Again

Becky Tilley, DVM, DACPV

Butterball, LLC, P.O. Box 10009, Goldsboro, NC 27530

Hurricane Florence is estimated to be the sixth costliest hurricane to hit in U.S. history in terms of property damage. Estimates total $44 billion in damage mostly due to heavy rainfall (up to 36 inches) resulting in flooding. The death toll attributed to Hurricane Florence stands at 53 in three states - North Carolina, South Carolina and Virginia. Estimates for crop damage and livestock losses to North Carolina agriculture industry exceed $1.1 billion. North Carolina Department of Agriculture reported livestock losses at 4.1 million poultry and 5,500 swine. This presentation will cover preparation prior to Hurricane Florence, events during the hurricane, and response to wind damage, flooding, and disease transmission resulting from the storm.

Hurricane Florence: North Carolina Department of Agriculture Response and Impact on NC Poultry Production

Michael P. Martin, DVM, MPVM, ACPV

NCDA&CS, Director, Poultry Division

The North Carolina Department of Agriculture and Consumer Services (NCDA&CS) responded in the immediate aftermath of Hurricane Florence with logistic resources and support to facilitate assessment of farms throughout the state. Additionally, NCDA&CS is engaged in efforts to properly manage disposal of poultry operations most impacted by Florence – approximately 4.2 million birds lost. Shortly after landfall, NCDA&CS successfully petitioned the Federal Emergency Management Agency (FEMA) for resources to assist farms with significant mortality to assist with the proper disposal of mortality and unusable litter. The proper disposal of this material protects not only public health, but also, the environment of North Carolina. Poultry farmers in North Carolina had many hardships to overcome because of this unprecedented weather event. Flood damage was pervasive after several waterways in the region reached 1000-year event levels of water inundation. The presentation will summarize the NCDA&CS response and highlight the impact on the commercial poultry industry in NC.

An underestimated tool: 25-Hydroxyvitamin D3 for intestinal and bone health for broilers raised without antibiotics.

Tina Yun-Ting Wang

DSM Nutritional Products North America-Animal Nutrition and Health

Chickens raised in a commercial environmentally controlled operation have limited access to natural sunlight, a critical component for the natural synthesis of vitamin D. Therefore, the addition of vitamin D3 to the diet is essential to fulfill nutritional requirements. In a 2-step process, vitamin D3 is first transformed into 25-Hydroxyvitamin D3 (circulating form) in the liver. This is followed by hydrolysis to the active form (1,25-dihydroxy vitamin D3) by 1α-hydroxylase enzyme found mainly in the kidney, but also present in the breast, thigh, intestine, and cecal tonsil. Transformation of vitamin D3 into 25-Hydroxyvitamin D3 could be severely limited by liver damage from stress, mycotoxins, viruses or bacterial infections. Hence, adding 25-Hydroxyvitamin D3 to animal diets can ensure sufficient precursor is present for the synthesis of the active form by bypassing the 1st step in the liver. Published research has shown vitamin D is critical for skeletal development, immune modulation, and intestinal health, as well as production variables such as hatchability and breast meat yield. Chickens raised without antibiotics (RWA) face increased challenges from coccidiosis, dysbacteriosis, and femoral head necrosis as opposed to broilers raised in a conventional program. Maintaining a healthy immune system is one of the keys for success in an RWA program. Therefore, this review focuses on the bone, immune system, and intestinal health benefits of feeding 25-Hydroxyvitamin D3 for RWA commercial broilers.

Dinner with Henrietta: Nutritional Issues in the Small Flock

Patricia S. Wakenell, Kristen Hill-Thimmesch, Laura Morman

Dept. of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, Purdue College of Veterinary Medicine, West Lafayette, Indiana

With the rising popularity of small flocks, particularly with first time owners, nutritional issues are increasingly being observed. This is particularly true with the variety of feed types, supplements, treats, and anecdotal information available on the internet. Many owners want to provide the best for their poultry, and this often involves purchasing feed that is attractive but nutritionally inadequate. In addition, there are abundant supplements and treats being marketed that may be harmful to the birds. Table scraps, particularly meat scraps, also contribute to issues that are uncommon in commercial flocks. I will present the more common issues that we have seen amongst the 500+ small flock farm visits that we have conducted over the

last 10 years. I will include the contributing reasons for each of the clinical scenarios presented.

Extension 3.0 in Backyard Poultry Flocks in California

Myrna Cadena[a], Teryn E. Girard[b] and Maurice Pitesky[a]

[a]UC Davis, School of Veterinary Medicine, Department of Population Health and Reproduction, Cooperative Extension, One Shields Ave, Davis, CA 95616, USA.
[b]University of Calgary, School of Veterinary Medicine, 2500 University Dr. NW Calgary, Alberta, Canada T2N 1N4

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

Providing Poultry Disease Education in Myanmar

Richard M. Fulton

Michigan State University Veterinary Diagnostic Laboratory And Pathobiology and Diagnostic Investigation College of Veterinary Medicine Michigan State University, East Lansing, Michigan 48824

February of 2018 a poultry disease workshop was held in Mandalay, Myanmar (formerly known as Burma) for the Myanmar Livestock Federation (Mandalay Region) for the Farmer-To-Farmer project of Winrock International. Participants consisted of two diagnostic laboratory veterinarians, seven poultry veterinarians, fourteen poultry farmers and three poultry farm managers. Topics of the training were Biosecurity Principles, How Poultry Fight Diseases, Vaccines and Vaccination, Poultry Diseases of Myanmar, Disinfectants and Their Use, Planning for Highly Pathogenic Avian Influenza and Introduction to the US Poultry Industry. Farm tours revealed that there were sources for improvement in the current biosecurity practices. Both meat and egg chickens were grown in open-sided houses on stilts over fish ponds. Many farms had farm workers living on site and those workers had dogs as well as their own flock of chickens including fighting cocks. The most common disinfectant for foot baths and for pathways leading to poultry houses was powdered lime. Based on discussions with the poultry farmers, the diseases reported to be in Myanmar were avian influenza, infectious bronchitis, infectious bursal disease, infectious laryngotracheitis, infectious coryza, pox, inclusion body hepatitis, *Ornithobacterium rhinotracheale* (ORT), E. coli, *Mycoplasma gallisepticum*, *Mycoplasma synoviae,* and Chronic Respiratory Disease (CRD) of broilers. Vaccines were available for most diseases from CEVA, Merial, and Fort Dodge.

Femoral Head Necrosis (FHN): Incidence and distribution in the U.S. broiler industry

Jaime Ruiz, DVM, MSc, MAM, Dipl. ACPV, Lynn Warren PhD.

Elanco Animal Health - 2500 Innovation Way, Greenfield, IN 46140 USA

Femoral Head Necrosis (FHN) is one of the most common leading causes of pathologic leg conditions in broiler chickens. We will review descriptive statistics coming from routine necropsy findings of this pathologic condition. Historical data will be analyzed to determine year, region, seasonal effects and age group distribution. Topic / category within the program: Epidemiology leg conditions. Program tags: Femoral Head Necrosis Leg conditions Elanco is a trademark of Elanco or its affiliates. © 2018 Elanco or its affiliates.

Role of wooden breast in late mortality in conventional broiler chickens and its prevalence prior to processing

Sesny Gall[a], Hannah M.L. Sather[a], M. Mitsu Suyemoto[a], A. Richard Sharpton[b], Luke B. Borst[a] H. John Barnes[a], and Rocio Crespo[a]

[a]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607;
[b]Perdue Farms Milling, 1342 Element Way, Apt 6106 Wilmington, NC 28412

"Wooden Breast" has emerged worldwide in conventional broiler chickens as an economically significant muscle disease. Grossly, affected breast muscle is hardened, rubbery, and paler than normal muscle. To our knowledge, the concerns surrounding wooden breast have been limited to meat quality and economic losses due to rejection by the consumer or condemnation of affected breast meat. In a previous study conducted in a small commercial broiler flock from a local integrator raised at the CVM, we observed a syndrome where broilers, during the 2 last weeks of the growing period, were unable to turn back onto their legs when they accidentally fell onto their backs ("turtle birds"). During the same period of time, the dead birds showed a high prevalence of wooden breast positively correlated with pulmonary congestion. We hypothesized that wooden breast is also an animal welfare concern as it predisposes to turtle birds. If not found and righted, turtle birds die of respiratory distress. In a similar flock that will be raised in January 2019, every turtle bird and a healthy bird (cohort bird) with similar weight chosen in the flock will be euthanized, necropsied and histologically scored for lung congestion and wooden breast. This will allow us to know the prevalence of wooden breast and lung congestion in dead birds, turtle birds, and in the overall flock. With these data, we will be able to confirm or reject our hypothesis about wooden breast being an animal welfare concern.

What's in the tracheal microbiome of a commercial broiler poultry flock?

Kelly A. Mulholland[a], Monique Robinson[a], Sharon J. Keeler[a], Timothy Johnson[b], Calvin L. Keeler, Jr.[a]

[a]Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716
[b]Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN 55455

Microbiomes are complex environments containing a variety of microorganisms including viruses, bacteria, yeast/fungi, archaea and protozoa. Disturbances to these environments may result in disease. Avian respiratory disease complex is an example of a multi-faceted syndrome that commonly effects the poultry industry. Interactions between a combination of pathogenic bacteria, fungi and viral infectious agents in the avian respiratory microbiome can lead to high mortality rates. Our first objective was to design an automated bioinformatics workflow and avian-specific viral genome database to rapidly detect viruses and quantify abundance and diversity. Our second objective was to aggregate all microbial data and provide a comprehensive analysis of the respiratory microbiome of a healthy commercial poultry flock. Tracheal samples were collected from

a healthy broiler flock at the day of placement and at weekly intervals throughout the seven week grow–out cycle. RNA and DNA were extracted and sequenced and DNAseq, RNAseq and 16s data was obtained. This data was analyzed utilizing the bioinformatics workflow and viral genome database we developed. As expected, a variety of RNA and DNA virus families were detected, including coronavirus, gyrovirus, and low levels of herpesviruses. Also, the diversity of the viral microbiome increased as the birds aged. Interestingly, a complex bacterial microbiome changed during growth of the flock. The bacterial microbiome could also be correlated with changes in the bacteriophage microbiome. By aggregating this data, along with information gained on the yeast and fungal microbiome, the development and evolution of the avian respiratory microbiome was determined.

Enhanced statistical process control systems for monitoring and predicting live operations performance, plant performance and pathogens

Isaac Duerr[a], Charles Corsiglia[b], Tim Buisker[c]

[a]Senior Data Scientist, Smart Data Science Solutions, 500 Hill St., Galena, IL 61036. [b]Manager of Veterinary Services, Foster Farms, 1000 Davis St., Livingston, CA 95334. [c]Principal Data Scientist, Smart Data Science Solutions, 500 Hill St., Galena, IL 61036.

Our objective is to augment Statistical Process Control (SPC) algorithms with machine learning techniques to build advanced SPC Systems capable of monitoring Key Performance Indicators and pathogen results in live operations and the processing plant. Based on a producer's historical data, SPC Systems determine if observed variation in an outcome is due to natural fluctuation or is indicative of a production problem or outside factor. Machine learning algorithms generate predictive Outcome Scores that guide decision making under uncertainty and allow sufficient lead-time to introduce changes in the production environment based on these predictions. The resulting SPC system was back-tested using historical data to confirm that it was capable of detecting short-term aberrations as well as incremental long-term changes to an underlying process. It was then implemented in a real production environment for >6 months and showed successful detection of more than 10 short-term events and 3 long-term shifts in underlying baselines across multiple production levels (breeder and grow-out) and regions. The predictive machine-learning algorithm was able to identify with 80% accuracy which flocks were most likely to have positive pathogen-related tests on the final (raw) outgoing product. A broad range of KPIs can be monitored: grow-out production parameters (e.g., weight gain, FCR, mortality), egg production, pathogen prevalence (e.g., biosecurity in live operations, food risk in raw product), and others. Accurate (>80%) predictions of some outcomes are possible with lead times of up to two weeks.

Pathology

Broiler Bone Evaluation: What is Normal and Potential Causes of Lameness

Frederic Hoerr, Floyd Wilson, Craig Wyatt, and Suzanne Dougherty

Pilgrims

The commercial broiler industry has focused on improving animal welfare with a focus on reduction of lameness and skeletal diseases including rickets, tibial dyschondroplasia and other mineral and vitamin deficiencies. Commercial broiler skeletal disease and lameness are influenced by production management, nutrition and genetic selection. While improvements have been made, we still don't have a complete understanding of the etiology and pathogenesis of lameness or what is normal in today's yielding broiler. The focus of these studies evaluated gross and histological parameters in broilers of both normal and abnormal gait scores. This presentation will focus on the history of the study, review gross findings and how nutrition and breed can influence lameness and disease. This will be part 1 of 2 presentations. The second presentation with cover histopathology findings.

Skeletal development in a broiler chicken dietary study: histopathology correlates of clinical findings

Floyd Wilson[a], Suzanne Dougherty[b], Craig Wyatt[c], and Frederic Hoerr[d]

[a]Mississippi State Veterinary Research Diagnostic Laboratory, Pearl, Mississippi
[b]Pecking Around Consulting, Madison, Alabama
[c]AB Vista, Stilwell, Kansas
[d]Veterinary Diagnostic Pathology, LLC, Fort Valley, Virginia

This study involved broiler chickens in 24 pens, representing 2 genetic strains and 6 diets. Femurs and tibias from the 12 experimental groups were examined histologically at 35 days of age. Bones were collected in neutral buffered formalin, decalcified, and then routinely processed for histopathology. Proximal femur was sectioned through the center of the femoral head. The stifle joint including distal femur, joint structures, and proximal tibia, sectioned through the median plane. Histologic lesions were identified and scored semiquantitatively for severity on a scale of 0 (normal) through 5 (severe). Histomorphometric evaluations were also done on the physis of the femur. A variety of acute or chronic and apparently traumatic injuries including microfractures and focal cortical fibrosis (or fibrous cortical defects, FCD) were observed. Two main histologic changes that showed some degree of variation in both the average severity and incidence in association with feed group were femoral microfractures and FCD. The difference in the expression of femoral microfractures demonstrated significant differences between some feed groups. Trending differences occurred for diet and cortical fibrosis. Microfractures were often associated with the FCD sites suggestive of predisposition by the fibrous defects. With mild variation, femoral growth plate morphometrics were not statistically different between feed treatments.

Myopathic lesions in broiler chicken heritage breeds and red junglefowl

Hannah M.L. Sather, Laura R. Chen, Luke B. Borst, H. John Barnes

College of Veterinary Medicine, North Carolina State University
Raleigh, NC 27607 USA

Wooden breast is an emerging muscle disease of broiler chickens characterized grossly by hard, pale breast muscle (*Pectoralis major*) and microscopically by myopathy, fibrosis, and perivascular lymphocytic infiltration. Modern broiler chickens were created by crossing Cornish and Plymouth Rock chickens, while the red junglefowl is the ancestor of all chickens. In this study, breast muscles from 24 five-week-old White Rock, Black Cornish, and red junglefowl were examined histologically for myopathy and lymphocytic infiltration. Myopathy and lymphocytic infiltration were identified in all 3 breeds, being significantly higher ($P<0.05$) in red junglefowl. Muscle lesions in broiler heritage breeds and red junglefowl show marked similarities to early wooden breast lesions in modern broilers. These findings suggest that wooden breast may be an exaggerated manifestation in modern broilers of myopathic conditions already present in their progenitors.

I See Liver Spots!

Claude Hebron

Prestage Farms, Inc.

A steady increase in liver granulomas has been noted in a commercial turkey operation in the southeastern United States over the past three years, with a more dramatic increase in 2016-2017. This increase has occurred in all strains and sex/weight categories. Liver granulomas are a concern due to poor performance of affected birds as well as complications in the processing plant. A number of factors have been identified as possible contributors to the increase. Changes in various

environmental and management parameters over the aforementioned time period will be discussed.

Food Safety

Salmonella Enteritidis Invasion of Internal Organs and Contamination of Eggs from Experimentally Infected Laying Hens of Four Commercial Genetic Lines

Richard K. Gast[1], Prafulla Regmi[2], Rupa Guraya[1], Deana R. Jones[1], Kenneth E. Anderson[2], Darrin M. Karcher[3]

[1]U. S. National Poultry Research Center, USDA Agricultural Research Service, Athens, Georgia 30605
[2]Department of Poultry Science, North Carolina State University, Raleigh, NC 29765
[3]Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

The prevalence of *Salmonella* Enteritidis in commercial egg-laying flocks is a public health concern because reproductive organ colonization in infected hens causes deposition inside eggs. Flock housing conditions can influence avian *Salmonella* infections, but the food safety implications are unclear. The present study assessed internal organ invasion and egg contamination by *Salmonella* Enteritidis (SE) in experimentally infected laying hens of 4 commercial genetic lines (2 white egg and 2 brown egg lines). Groups of hens from each line were housed at similar stocking densities in both conventional cages and enriched colonies. All hens were orally inoculated with 107 cfu of SE. In 2 trials, portions of 5 internal tissues were removed at 1 wk post-inoculation and cultured to detect SE. In 2 other trials, eggs laid 5-24 d post-inoculation were cultured for the pathogen. Significant ($P < 0.05$) differences in SE isolation from intestinal samples and eggs were seen between individual hen lines. The overall SE recovery frequencies from intestinal samples (69.5% vs. 43.8%) and eggs (3.4% vs. 1.6%) were significantly greater for the white lines combined than for the brown lines. No line or housing differences were seen for other organs. One brown line yielded greater intestinal SE isolation in conventional cages than in enriched colonies, but housing did not affect egg contamination frequencies for any line. These results demonstrate that SE colonization of the intestinal tract and deposition in eggs can vary between genetic lines of egg-laying hens. However, egg contamination was not influenced by different housing systems.

Effect of yeast derived feed additive on *Salmonella* and antibiotic resistance

M. M. Kromm[1] and T. J. Johnson[2]

[1]Jennie-O Turkey Store, Willmar, MN
[2]Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN

There are an overwhelming number of “alternative” in-feed additives available on the marketplace with claims to reduce *Salmonella* in birds. In addition, a few also claim to have “restorative” effect on antibiotic sensitivity. Data related to *Salmonella* as well as genotypic and phenotypic antibiotic resistance will be presented. Flocks were fed a yeast-origin product alone, BMD alone, or BMD + yeast-origin product. No significant differences were found between the various treatment groups for either *Salmonella* related or antibiotic resistance-related measurements.

Sex and the Single Salmonella: What We Don't Know Hurts Us

Jean Guard[1], Devendra Shah[2]

[1]US Department of Agriculture, [2]Washington State University, Pullman, WA

Reducing Foodborne Illness and protecting consumers is a complex endeavor requiring careful economical implementation of research and regulations to facilitate detection, intervention, and prevention. Using Salmonella enterica subspecies I as a model organism, a formula for evaluating where research dollars can be used to fund novel lines of inquiry is presented. The formula also provides a shared framework by which current but dissimilar research can be interconnected to guide the

efficacious application of results to solve real world problems. The formula, in simplest terms, states that foodborne illness (FI) is the sum function of environmental opportunity (EO), and the bacterial properties of genetic change (GC), clonal expansion (CX) and virulence factors (VF). However, the formula also includes the concept of flow to illustrate connection and exchange between compartments. The formula is purposefully malleable so that it can be refined as insights into its components are incorporated over time. The basis for the formula was developed by application of the ISR serotyping approach, which genetically uncouples serotype from methods based on O- and H-antigen genes and surface epitopes. Comparison of ISR sequences to the entire NCBI database for subspecies I genomes (November 2018, approximately 943 genomes) indicated that serotypes Typhimurium and Enteritidis, which are the two most prevalent causes of foodborne salmonellosis in the world, have a relatively high index of homologous recombination (HR) compared to other serotypes. Thus, these results suggest that HR is a contributor to the compartment of genetic change (GC) that could contribute to the epidemiological impact of some major serotypes. A literature review indicated that HR is not well investigated for its role in propagating salmonellosis. Another application of the model is to provide a basis for understanding diversity in Salmonella enterica subspecies I, which has over 1500 serotypes with only 2% being frequent contributors to foodborne illness.

Immunization with live *Salmonella* vaccine, programs in broiler chickens and resulting impact in plant samples

Robert Evans

Elanco

Contamination 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. Hassan and Curtiss (1996) demonstrated that vaccination of hens with a live, attenuated *S*. Typhimurium (ST) strain induced long-lasting *Salmonella*-specific IgG antibodies that were transferred into eggs. Progeny from vaccinated hens infected at 1 day of age with wild-type ST showed a significant decrease in the prevalence of the wild-type ST isolated from organs and the intestinal tract compared to chicks from non-vaccinated hens (Hassan and Curtiss, 1996). Live and killed vaccines, when used together, 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. Commercial broiler production vaccination programs to reduce *Salmonella* were evaluated by obtaining chicken parts rinsates to determine the prevalence and population distribution of *Salmonella*. Plant interventions were unchanged during the observation periods and the effectiveness of the vaccine programs was determined by reduction in the prevalence of *Salmonella,* specifically in serogroups B and D. This presentation will detail the specific programs, relative outcomes and effect of alterations within a program as pertains to reducing *Salmonella* prevalence and the serotype profile of parts rinsate cultures.

Live Salmonella Typhimurium Vaccination Lowers the Load Of Various Salmonella Serogroups at Different Challenge Ages in Broilers

Manuel Da Costa

Zoetis

The presentation will focus on efficacy of live salmonella vaccines in broiler chickens against various salmonella serotypes at different ages.

Use of *Salmonella* vaccines in commercial turkeys for pre-harvest reduction in *Salmonella* load at processing

M.E. Lighty

Jennie-O Turkey Store, Willmar, MN 56201

Salmonella contamination of ground turkey products has become of increasing concern to the turkey industry in recent years. Various processing plant interventions have been successful at reducing the load of *Salmonella* in ground products, but these interventions have not completely eliminated the risk of food-borne *Salmonella* infections. Increasing emphasis has been placed on pre-harvest interventions to reduce the load of *Salmonella* in birds coming into the processing plant. The use of two different commercially-available live attenuated *Salmonella* Typhimurium vaccines were evaluated in commercial tom turkey flocks for their effect on load of *Salmonella* in the barn environment as well as final ground product at the plant. Multiple vaccine schedules were evaluated to identify the optimal schedule for maximum reduction of *Salmonella* load. Administration of a single-dose per bird of a live attenuated S. Typhimurium vaccine at 3, 6, 9, 12, and 15 weeks of age resulted in a reduction in *Salmonella* load on pre-market environmental bootie swabs. Observations were also made on the influence on *Salmonella* serotypes present in the environment. Results of additional vaccination schedules will be also discussed.

Most effective form of vaccination application using a live attenuated *Salmonella typhiumurium* vaccine

J. Tourville

Jennie-O Turkey Store, Willmar, MN 56201

Prevention of clinical salmonellosis in young turkeys is important. Every opportunity to prevent morbidity and mortality in flocks is essential, which also reduces the need for antibiotic use. Effective application of vaccination is an important tool in prevention of clinical salmonellosis. Three routes of application were evaluated for vaccine uptake. Flocks received an initial application of a live attenuated Salmonella typhiumurium vaccine at placement via one of three routes: drinking water, gro-gel, or a coarse spray. All flocks were then boosted through drinking water at day 21. Results indicate vaccine application was optimal when applied either through drinking water or coarse spray.

Feed and drinking water delivered chitosan-subunit *Salmonella* nanovaccine for poultry

Sankar Renu[a], Yi Hana, Yashavanth S Lakshmanappa[a], Santosh Dhakal[a], Nicole Irizarry Tardi[a], Ninoshkaly Feliciano-Ruiz[a], Shristi Ghimire[a], Ramesh Selvaraj[b], Gourapura J Renukaradhya[a]

[a]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691, USA, and Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA. [b]Department of Poultry Science, University of Georgia, Athens, Georgia, 30602.

Salmonellosis in poultry is a serious economic issue. The major concern is the public health hazard caused by consumption of *Salmonella* contaminated poultry products. Vaccination in poultry is the available choice to mitigate *Salmonella*, but all the available commercial *Salmonella* inactivated vaccines have to be injected manually to each bird and thereby making it practically very difficult to

farmers and stressful to birds, especially in large poultry farms. Thus, we formulated subunit antigen (SAg) loaded surface flagellar (F) protein coated mucoadhesive chitosan nanoparticles (CS NPs) (SAg-F-CS NPs) for oral feed and drinking water based delivery in poultry. Formulated SAg-F-CS NPs had average particles size distribution of 514 nm, polydispersity index of 0.3 with positive charge and spherical in shape. Specific fluorescent dye tagged surface F-protein decorated CS NPs were efficiently taken up in the chicken immune cells by *in vitro* and target ileal immune cells by *in vivo* studies. The SAg-F-CS NPs oral feed and drinking water based vaccinated and *Salmonella* challenged birds enhanced antigen specific secretory and mucosal IgA antibody responses, associated with increased proliferation of splenocytes. As well, SAg-F-CS NPs oral feed and drinking water based vaccination non-significant and significantly reduced challenge bacterial burden in chicken cecum, respectively. In conclusion, oral feed and drinking water based SAg-F-CS NPs vaccination enhanced immune responses associated with reduced bacterial infections and likely would be an effective candidate vaccine to mitigate *Salmonella* in poultry.

Evaluation of Long-Term Immunity and Protection against *Salmonella spp* by an Orally Administrated Subunit Vaccine

Maritza Tamayo[2], Emanuel Gumina[2], Ariel Sugezky[2], Sherry Layton[1,2]

[1]BV Science, 8651 Highway N, Lake St Louis, MO USA
[2]Vetanco SA, Chile 33, Buenos Aires, Argentina

Salmonella is the leading cause of foodborne infections and is a major public health concern worldwide. In this study we evaluated the efficacy of a commercially available universal inactive, orally administered, subunit vaccine against mobile *Salmonella spp*. (BTVS) and determined if the immune response was protective and persistent in two commercial layer flocks (n=120,000/lot). Pullets were given 2 doses of BTVS administered at d3 and 16 post-hatch; the third dose was administered in Lot 1 at d91 post-hatch and in Lot 2 at d84 post-hatch. Intestinal mucosal scrapings and serum were collected from 15 birds at 6, 21, 33, 67 and 89-weeks post hatch in Lot 1 and 5, 13, 22, 56, 78 and 120-weeks post-hatch in Lot 2. BTVS vaccine specific mucosal immune response (sIgA) and systemic immune response (IgY) was evaluated by proprietary ELISA and S/P ratios calculated to determine specific response. Results show that at all sample collection points there was significant ($p < 0.05$) sIgA and IgY/IgG antibody production that persisted for the duration of the field trials. Additionally, in Lot 2 at the end of the field trial (week 121) birds from vaccinated and unvaccinated flocks (n=20/flock) were challenged with *Salmonella enteritidis* (SE) (1x1010 cfu/bird). Significant differences were observed in SE recovery between vaccinated (20%) and non-vaccinated (65%) groups 10 days after challenge. This study provides evidence that vaccination with BTVS induces a strong mucosal and systemic immune response and protects poultry from *Salmonella* spp infection. Furthermore, this response provides long-term immunity.

Reovirus

Genotypic classification of avian reoviruses

Holly S. Sellers, Samantha L. Day and Erich G. Linnemann

Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens, Georgia, 30602, USA

Diagnosis of avian reoviruses from clinical cases of tenosynovitis is achieved by virus isolation and RT-PCR amplification of the viral genome. Characterization of reoviruses relies on amplification of the Sigma C encoding region of the reovirus S1 gene followed by sequence analysis. To date, up to 6 genotypes have been reported. Phylogenetic analysis reveals significant differences between the genotypes but also within the genotypes. Recent reovirus isolations in our laboratory identified reoviruses that did not belong to any of the 6 previously described genotypes and thus were identified as a new genotype 7. Due to the high degree of variability within several previously

described genotypes, further designation of new genotypes is supported to provide more concise results for use by clients.

Evaluation of the Pathogenicity of Recent Reovirus Field Isolates in Genotypes 2, 4 and 6 in Commercial Broilers

Angela Michelle Stewart, Holly S. Sellers, and Erich Linnemann

Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia Athens, Georgia, 30602, USA

Globally, reovirus is ubiquitously found in commercial poultry operations. Many strains of reovirus are nonpathogenic, however pathogenic variant strains are becoming more prevalent in poultry flocks across the United States. Since 2011, increased incidences of tenosynovitis from variant field isolates have been detected in chickens and turkeys. The pathogenicity of recent reovirus field isolates belonging to genotypes 2, 4 and 6 were evaluated. Seventy-five chicks were divided into 6 groups of 10 chicks and 1 group of 15 chicks. Each group were either inoculated via the foot pad or orally gavaged. Body weight measurements and hock/tendon measurements were recorded every 7 days over a 28-day time period. Serum was collected at one-day of age from the progeny and was tested by virus neutralization, all samples tested negative for antibodies against reovirus from genotypes 2, 4 and/ or 6. Grossly, the hocks were markedly swollen, and a few were hemorrhagic, some hearts had a jelly like consistency. Hearts, thymus, bursas, small intestines and tendons were submitted for histopathology. Histological results are currently pending.

Cold adaptation and evaluation of an avian reovirus genotype one variant

William Dawe[a], Erich Linnemann[a], Vanessa Gauthiersloan[a], Holly S. Sellers[a]

[a]Poultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA

Avian reoviruses are causative agents of tenosynovitis and viral arthritis. Commercially available reovirus vaccines do not protect against challenge with emerging variants associated with tenosynovitis and lameness. Reovirus field isolates are commonly included in autogenous vaccines, but the protection provided by the inactivated autogenous vaccines, in the absence of a homologous live prime, may have an insufficient duration of immunity. In this study, a genotype one variant field isolate was serially passaged in LMH cells at 32℃ to evaluate of cold adaptation as a method for attenuation of variants. The emergence of a cold-adapted phenotype was assessed by in vitro replication kinetics of the wild type and cold passaged isolate at 32, 37, 39, and 41℃. Evaluation of reovirus attenuation methods can assist in the development of live commercial vaccines that are needed to increase the spectrum of reovirus immunity provided by vaccination programs.

Horizontal Transmission of Reovirus and Age-Associated Susceptibility to Development of Viral Arthritis/Tenosynovitis

Milos Markis, John K. Rosenberger, and Sandra C. Rosenberger

AviServe LLC, Delaware Technology Park, 1 Innovation Way, Ste 100, Newark, DE 19711

Reovirus infections and associated diseases continue to be an economically-significant factor for U.S. broiler chicken producers. Currently the most important reovirus-associated disease in broiler chickens is viral arthritis/tenosynovitis. Since 2011 chicken reoviruses have continued to shift antigenically, which complicates vaccine-based

control strategies. One of the ways reoviruses change is through reassortment of genome segments when different genotypes of reovirus co-infect chickens. Reovirus reassortants can be transmitted horizontally and vertically to susceptible chcikens. A model of *in ovo* reovirus infection was developed and findings presented in 2018. Subsequently a reovirus "shedder" model was developed in which *in ovo*-infected chickens were used to disseminate reovirus to susceptible chickens horizontally through contact. Pathogenesis was evaluated in horizontally-infected chickens and compared to *in ovo*-infected chickens. Age-associated resistance to development of viral arthritis/tenosynovitis has been reported. Here, the "shedder" model was utilized to re-evaluate the age-associated resistance to development of viral arthritis/tenosynovitis when chickens were exposed to reovirus through shed, which mimics natural exposure.

My Experience with Laboratory Diagnosis of Reovirus Tenosynovitis

Tahseen Abdul-Aziz

Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture and Consumer Services, Raleigh, North Carolina, USA

For the last few years, broiler chickens have been received at North Carolina Veterinary Diagnostic Laboratory System because of "leg problem". Clinical history indicated that birds in the flock sit and cannot stand and need to be culled. As high as 10% of the flock is affected. Most of the submissions were broilers over 40 days of age, but the disease was diagnosed in broilers as young as 14 days. In multiple-house farm, not all houses were affected and, and in some cases only one of several houses on a farm as involved. Diagnosis protocol included ante-mortem examination, collecting blood samples for reovirus ELISA, humane euthanasia of the birds, post-mortem examination, histopathology on digital flexor/gastrocnemius tendon, and, in some case, virus isolation. On ante-mortem examination, classically, affected birds were sitting on one leg or in sternal recumbency with one leg extended laterally or backward. In some birds, there were variable degrees of swelling of one or both tibiotarsi, particularly over the area of digital flexor tendons on the anterior aspect of the bone. Swelling of one or both joints was present in some birds but was not a consistent feature. The spleen was mildly enlarged in some birds. Histopathologic lesions consisted of lymphoplasmacytic tenosynovitis of digital flexor tendons and gastrocnemius tendon with hyperplasia of synovial cells. Heterophilic infiltrates of varying severity were present in some cases. For ELISA, positive or negative results are determined by sample-to-positive (SP) ratio. The serology results were always interpreted in conjunction with the clinical disease and gross and histopathologic lesions. The ELISA were found to be very useful in the diagnosis of the diseases when used in conjunction with other ancillary test.

Virology

Immune Responses Induced in Chickens by a Genetically More Homogeneous Infectious Bronchitis Virus Vaccine

H. Toro, V. van Santen, R. Zegpi, A. Mishra

Auburn University

We previously demonstrated that adaptation of an extensively used embryo-attenuated IBV ArkDPI-derived vaccine to chicken embryo kidney (CEK) cells shifted the virus population towards homogeneity in spike and non-structural protein genes. More importantly, the typical ArkDPI vaccine virus subpopulations commonly emerging in chickens vaccinated with the commercial ArkDPI vaccines were not detected in chickens vaccinated with the CEK cell-adapted Ark virus (CEK-Ark). In addition, CEK-adapted ArkDPI vaccination drastically reduced the emergence of subpopulations from a wild Ark challenge strain. We hypothesize that vaccination with the more homogeneous CEK cell-adapted virus eliminates emergence of novel viruses after Ark challenge because it elicits distinct immune responses (in quality or quantity), which differ from immune responses elicited by the genetically diverse commercially available embryo-attenuated ArkDPI vaccine. Understanding the immune

responses induced in chicken populations to more homogeneous and stable IBV vaccine viruses is the basis to allow global procedures aimed at reducing genetic variability, not only in ArkDPI-type vaccines, but also in all other available serotype-specific IBV attenuated vaccines.

Broiler Study Evaluating Arkansas IBV Protection Achieved By Various Heterologous IB Vaccine Combinations

Kalen Cookson[1] , Manuel Da Costa[1], John Dickson[1] and Jon Schaeffer[1]

[1]Zoetis US Poultry, Durham, NC

A broiler study also being presented at this year's meeting demonstrated that co-administering either Mass or GA98 with GA08 vaccine significantly enhanced cross protection against a contemporary 1639 isolate (DMV/240/18). While adding Ark to GA08 did improve protection nearly significantly (p=0.061), non-challenged vaccinates also demonstrated more tracheitis and vaccine persistence than the other treatments. To explore the feasibility of forgoing Ark vaccine in broiler programs, this study compared various heterologous bivalent combinations against an Ark IBV challenge. 450 day of age broilers were divided into 5 groups of 5 isolators each and vaccinated with live B1 and the following IB vaccine(s): Ark, GA08+GA98, GA98+Mass, GA08+Mass and None. Choanal swabs were taken weekly from vaccinated controls. At 25 days of age, 3.5 EID50 Ark IBV challenge was given via eye/nose drop. At 30 days of age, all birds were bled, evaluated for clinical signs and scored for internal lesions; tracheas were swabbed for IBV PCR and preserved in formalin for histopathology. Using the "VI negative" Ct≥35 cut-off, challenge controls were 95% positive while Ark vaccinates were 85% protected. GA08+GA98 only gave 29% protection but Mass with either GA98 or GA08 gave 50% and 48% protection, respectively. Using the "significant load" Ct≤30 cut-off, 80% of controls hit this mark while all vaccine treatments gave over 80% protection. Ark and Mass+Ga98 gave 100% protection followed by Mass+GA08 (90%) and GA08+GA98 (81%). Statistical analysis will be presented along with other study results which were still pending at time of abstract submission.

False Layer Syndrome Caused by Infectious Bronchitis Virus, Genetic Characterization and Pathobiology Insights

Rodrigo A. Gallardo, Alexandra Mendoza-Reilley, H.L. Shivaprasad, Ivan Alvarado, Corinne Giroux, Holly Sellers

University of California, Davis

In order to better understand what makes this virus different from other IBV strains and why generates reproductive pathology in affected flocks, we molecularly characterized two viruses associated with this syndrome. In addition, we designed a trial to understand tropism, viral load in tissues and microscopic pathology in SPF chicks infected with two different doses (high and low) of one of the viruses. Molecular biology, viral tropism, viral load and microscopic pathology outcomes will be discussed.

Use of different NDV ELISA kits for the evaluation of the antibody response at different ages elicited by a recombinant Newcastle disease vaccine (HVT+ND) applied *in ovo* in both specific pathogen free (SPF) birds and commercial broilers.

Osorio, C., Smith,D.,Slacum, G., Banda, A., Orozco, E.

Boehringer-Ingelheim

The aim of this work is to evaluate the antibody response elicited by a recombinant Newcastle disease vaccine (HVT+ND) using four different kits of enzyme-linked immunosorbent assays (ELISA). At 18 days of incubation, both commercial broiler and specific pathogen free (SPF) chicken embryonated eggs were manually vaccinated by *in-ovo* route with a full dose of either HVT or HVT-ND vaccines. Upon hatching, the birds were housed in poultry isolation units, and assigned into four groups, a) broilers vaccinated with HVT-ND; b) broilers vaccinated with HVT; c) SPF birds vaccinated with HVT-ND, and d) SPF birds vaccinated with HVT. At one, two, three,

four and five weeks of age, blood samples were collected and the serum was separated to run ELISA tests to quantify antibodies against Newcastle disease. Birds vaccinated with HVT-ND developed humoral response detected by ELISA. The differences in the ELISA titers obtained by the three different kits were analyzed by statistical methods.

Host adaptation affects the transmission of virulent Newcastle disease viruses in chickens

Helena L. Ferreira†[a,b], Tonya L. Taylor†[a], Kiril M. Dimitrov[a], Claudio L. Afonso[a], David L. Suarez[a]

[a]Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, ARS, USDA, Athens, Georgia, USA.
[b]University of Sao Paulo, ZMV- FZEA, Pirassununga, 13635900, Brazil
† Authors with equal contribution

Virulent Newcastle disease viruses (vNDV) often cause disease in poultry, and some are known to be maintained in non-poultry species. The present study aimed to evaluate the potential risk of eight different vNDV to infect and cause disease in chickens and transmit to naïve birds. Six of the studied viruses were isolated from chickens, one from a cormorant, and one from a pigeon. Three-week-old, SPF chickens were divided into three groups per virus. Each group of five birds received a low, medium, or high dose of the respective vNDV by the oculonasal route. Three naïve birds were added to each group at 2 days post-inoculation (dpi). Virus shedding was quantified from swabs (2, 4, and 7 dpi), and seroconversion was evaluated at 14 dpi. All inoculated and contact birds in the chicken-origin vNDV groups succumbed to infection, except for two low dose groups. The cormorant virus infected group had neurological signs with mortality in birds receiving medium and high doses, but the birds from the low dose group were not infected. No clinical signs were observed in the birds inoculated with the pigeon-origin virus at any dose. All chickens displaying clinical signs shed virus in high titers, but cormorant and pigeon viruses shed low or no virus. A few directly-infected, surviving birds from high and medium dose cormorant and pigeon virus groups seroconverted. Overall, chicken-adapted viruses appear to infect chickens and readily transmit to naïve birds, whereas the viruses of non-poultry origin did not transmit to contact birds although they infected the chickens inoculated with the high dose.

Molecular-epidemiological and evolution study of the Newcastle disease virus causing outbreaks in California in 2018

Kiril M. Dimitrov[a], Mia Kim Torchetti[b], Mary Lea Killian[b], Tod P. Stuber[b], Beate M. Crossley[c], Nichole Hines Bergeson[b], Claudio L. Afonso[a], David L. Suarez[a]

[a]Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, US National Poultry Research Center, Agricultural Research Service, USDA, 934 College Station Road, Athens, Georgia 30605, USA
[b]National Veterinary Services Laboratories, Veterinary Services, U.S. Department of Agriculture, Ames, Iowa, USA
[c]California Animal Health and Food Safety Laboratory system, Davis Branch, University of California, Davis, California, USA

Newcastle disease is caused by virulent strains of Newcastle disease virus (vNDV) and is considered a foreign animal disease in the United States. This disease can be devastating as it causes up to 100% mortality in naïve birds and significant economic losses accumulate outbreak eradication and trade restrictions. In May of 2018, vNDV was detected in backyard exhibition chickens in southern California, and since, it has affected over 180 backyard flocks in four counties within the state. Comprehensive phylogenetic analyses revealed that the virus causing the outbreak belongs to sub-genotype Vb and is related to viruses whose circulation has historically been geographically limited to Central with sporadic introduction into California and other US states. The virus causing the 2018 California outbreak is genetically closest to a 2018 chicken isolate from Guatemala, but the 0.8% nucleotide distance between both viruses suggests no direct epidemiological relation. Still closely, but less related, were chicken isolates obtained in California, Belize and California in 2002, 2008, and 2008,

respectively (97.9-99.2% nucleotide identity). The 2018 California vNDV was even more distant (6.5%-10.2%) from the virulent viruses from sub-genotypes Vc (affecting poultry in Mexico, 2004-2017) and Va (maintained in cormorant species in North America). Molecular clock analysis estimated that the 2018 California and Guatemala viruses evolved from an unknown common ancestor that existed during the second half of 2015. In addition, next-generation sequencing was performed on the isolates from the 2018 California outbreak to facilitate the study of their evolutionary dynamics, single nucleotide polymorphism and variant analyses.

Evaluation of protection against AL-2 like Infectious Bursal Diseases Virus using a dual recombinant HVT-ND-IBDV vaccine along and in combination with a live 89/03 strain and two plaque intermediate standard strain of IBDV vaccine

Andres Montoya

Merck Animal Health De Soto, KS

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immnodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV are endemic in the southeastern United States. IBDV variant AL-2 (AL for Allen Laboratory) originated from Delaware in recent years has showed to affect broilers between the ages of 20 to 30 days or older showed moderate to severe bursal atrophy. Because IBDV variant AL-2 isolation in commercial broilers has increased in recent years in the southerner of the United States causing performance issues and the lunch of new vaccine technology of the first dual recombinant HVT-ND-IBDV vaccine an evaluation of the protection against AL-2 variant along or in combination with two live vaccines will be present.

Study of pathogenesis of Infectious Bursal Disease Virus (IBDV): Comparison between a South American Variant and a Classic Strain

Ariel E. Vagnozzi[a], A., Gonzalo Tomas[b], Gabriela Romero[a], Pablo Sansalone[c], Ana Marandino[b], María Isabel Craig[a], Silvina Pinto[d], Ruben Perez[b] and Udi Ashash[c].

[a]Instituto de Virología, Instituto Nacional de Tecnología Agropecuaria, Hurlingham (1686), Buenos Aires, Argentina.
[b]Instituto de Biología, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo (11400), Uruguay.
[c]Phibro Animal Health Corporation.
[d]Patología, Facultad de Cs Veterinarias, Universidad de Buenos Aires, Av. San Martín 5285, CABA (1417), Argentina.

Classic vaccines have been largely used to control infectious bursal disease virus (IBDV) in Argentina; however, IBDV is frequently detected in chicken farms. This situation is mostly due to a newly reported IBDV lineage, a South American (SA) variant. Since Argentinean flocks are extensively vaccinated with classic (CS) vaccines, we propose that the SA variant presents some attribute that allows it to infect immunized chickens. In order to verify if the adaptive advantage of the SA variant resides on its virulence we performed a study of pathogenesis comparing the dynamics of infection of both IBDV variants, SA and CS. In this study 108 SPF chickens were organized in 3 groups of 36 chickens each named SA, CS and negative control (C-). The SA and CS were inoculated with the correspondent IBDV variant (same dosage) while the C- remained non-inoculated. Samples of the spleen (S), bursa of Fabricious (BF) and cecal tonsil (CT) were taken on days 1, 2, 4, 7, 14 and 21 post-inoculation (dpi) and evaluated: *i*) S and BF weight ratio; *ii*) microscopic lesions of BF; and *iii*) viral load of BF, S and CT by RT-qPCR. The results did not show sustained significant differences in the evaluated variables at all time-points. In conclusion, no clear difference in virulence of SA compared with a field CS strain has been detected in this study. Further studies analyzing antigenic aspects of this variant

should be done to gain a better understanding of the presence of this strain among chicken flocks.

Cell-mediated Immune Responses in the Eye Associated Lymphoid Tissues of Chickens after Vaccination or Infection with Infectious Laryngotracheitis Virus (ILTV)

Maricarmen García[1], Gabriela Beltrán[1], David J. Hurley[1], Leah R. Read[2], and Shayan Sharif[2]

[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens GA. 30605.
[2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Local cell immune responses elicited by infectious laryngotracheitis virus (ILTV) after ocular stimulation with the CEO vaccine and the virulent 63140 strain was investigated. Dynamics of lymphocyte populations, assessment of MHC class I and MHC class IIHi expression, and transcription of cytokines genes that favor the cell-mediated immune responses were assessed in conjunctiva associated lymphoid tissues (CALT) and Harderian gland (HG). This study confirmed the essential role of the local cell mediated immune response to disease resistance and provided a first glimpse that ILTV can circumvent the local immunity by delaying innate responses and downregulating MHC class II expression.

Interpretation of Real-time PCR Data from Clinical Specimens Suspected for Vaccinal Laryngotracheitis Virus Infection.

Arun B. Kulkarni,

Georgia Poultry Laboratory, 3235 Abit Massey Way, Gainesville, GA 30507

Infectious laryngotracheitis (ILT) is an upper-respiratory disease of poultry with a world-wide distribution. Outbreaks of the disease results in severe economic losses due to moderate mortality and elevated morbidity. Molecular amplification of the glycoprotein C gene by real-time polymerase chain reaction as well as subsequent confirmation by histopathology remain the standard methods for the rapid diagnosis of LTV. We evaluated the correlation of PCR cycle threshold (Ct) value with the presence of histopathological lesions for 70 cases of vaccinal LT received by our laboratory in the past four years. This assessment indicated that low cycle threshold (Ct) values, typically up to 23, showed high correlation with histopathological lesions. Whereas cases with histopathological inconclusive lesions yield Ct values from 24-30. We also noticed that tracheal specimens from backyard flock showing the signs of respiratory illness yield high Ct values in the absence of characteristic intranuclear inclusion bodies. The quality of clinical specimen and the severity of virus infection are variable in field submissions. This poses a problem while comparing the Ct values from different clinical specimens. In order to understand the relationship between the severity of the disease in terms of histopathological lesions and virus load in the tissues, we determined the gene copy numbers from the archival DNA samples from clinical cases showing wide range of Ct values with or without LTV-specific microscopic lesions. In a separate experiment, Ct values and gene copy numbers from tracheal swabs from recently vaccinated broiler breeders were determined to assess the shedding of virus in CEO or TCO vaccinated flocks after moving to the hen house or the processing plant. Results from clinical observations and virus detection studies will be presented.

Efficacy of InnovaxÒ-ILT (recombinant HVT-LT) vaccine when administered alone or in combination with a chicken embryo origin (CEO) vaccine

Daniel Maekawa[A], Sylva M. Riblet[A], Linnea Newman[B], Rik Koopman[B], Taylor Barbosa[B] and Maricarmen García[A]

[A]Poultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602
[B]Merck Animal Health, Madison, NJ 07940

Infectious laryngotracheitis (ILT) is an acute respiratory disease of poultry that is mainly controlled through vaccination with live-attenuated and recombinant vaccines. ILT live attenuated vaccines are capable to protect chickens against clinical signs, mortality, and halt replication of the challenge virus. However, the live attenuated vaccines in particular the chicken embryo origin (CEO) retain inherent virulence and induce vaccination reactions associated with production penalties. Recombinant Herpesvirus of Turkeys (rHVT) - LT vaccines do not regain virulence but are less effective to halt virus replication after challenge. The objective of this study was to assess the effectiveness of a combined vaccination strategy in specific pathogen free (SPF) chickens. The InnovaxÒ-ILT vaccine was administered at 1 day of age subcutaneously followed by CEO vaccination administered at 6 weeks of age via eye-drop or drinking water. Chickens vaccinated solely with CEO via eye drop develop transient conjunctivitis which was not observed in rHVTLT + CEO vaccinated group of chickens or when the CEO vaccine was administered via the drinking water. The CEO vaccine genome load in the trachea of rHVT-LT + CEO vaccinated group of chickens was significantly lower compared to chickens vaccinated with CEO alone. After challenge significant reduction in clinical signs was observed in all vaccinated groups of chickens. Challenge virus genome load reduction of 89.0% to 94.4% was achieved in the trachea of CEO and rHVT-LT + CEO vaccinated groups of chickens, while a 63.5% reduction rate was observed for rHVT-LT vaccinated group. Challenge virus transmission to contact naïve chickens was only detected in the rHVT-LT group of chickens. Therefore, the rHVT-LT + CEO vaccination strategy reduced viral shedding after CEO vaccination and after challenge ameliorating the inherent efficiency weaknesses of either vaccine when administered by itself.

The role of vaccination on transmission of Marek's disease virus in poultry

John R. Dunn[1], Hans H. Cheng[1], Andrea Doeschl-Wilson[2]

[1]USDA – ARS Avian Disease and Oncology Laboratory, East Lansing, MI
[2]Roslin Institute, University of Edinburgh, UK

Marek's disease (MD) is currently controlled through biosecurity, widespread vaccination, and selection for genetic resistance. Although prevalence of MD is currently low in many parts of the world, history has repeatedly shown that Marek's disease virus (MDV) field strains have undergone multiple shifts of increased virulence that required introduction of new vaccines. This cycle of virus evolution followed by introduction of new vaccines is not sustainable in this large, expanding, and highly concentrated industry. In this study, we examined the potential role of vaccination in reducing quantity and duration of viral transmission, with the goal of reducing environmental virus load and thus increase the efficacy of existing and future control measures. First, we determined that 4 hours of exposure time to MDV-infected donor birds was sufficient to transmit infection to naïve recipient birds. For subsequent experiments, we used a donor-recipient challenge model to determine when, how much, and how long MDV was transmitted. Donor birds differed by vaccination status using turkey herpesvirus (HVT) vaccine and recipient birds were all highly susceptible. Our results indicate that vaccination has a significant effect on delaying the initiation of virus transmission. Next steps will be to determine which specific classes of MD vaccines are most effective at reducing environmental virus load for maximizing future control measures.

Are birds expressing Cas9 and guide RNAs against Marek's disease virus (MDV) resistant to MDV challenge?

Karel A. Schat[1], Arjun Challagulla[2] and Timothy Doran[2]

[1]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
[2]Australian Animal Health Laboratory, CSIRO, Geelong, Vic, Australia

The CRISPR/Cas9 gene editing system has been used by us to show that transfected cells expressing Cas9 and three specific guide (g)RNAS against the immediate early ICP4 gene of Marek's disease virus (MDV) inhibit MDV replication in vitro. Last year we reported that we had generated the first transgenic chickens expressing the ICP4-specific gRNA and transgenic chickens expressing Cas9. These birds will crossed to obtain chickens expressing both gRNA and Cas9 as well as control birds only expressing gRNA or Cas9 or lacking both. Three experiments are planned to test the hypothesis that chickens expressing gRNA+Cas9 are resistant to challenge with pathogenic MDV using the Australian Woodlands strain. In the first experiment we will inoculate the 4 groups of chickens with MDV and collect samples for virus isolation and qPCR analysis between 6 and 18 days post inoculation. In the second experiment we will repeat the first experiment with the addition of non-infected contact chickens to determine if we can repeat the results of the first experiment and to exclude the possibility of horizontal transmission. In the third experiment we will use control shedder chickens to expose the transgenic chickens to the normal route of infection. The transgenic chickens will be examined for virus replication between 6 and 18 days post exposure. The results of these experiments will be reported.

Next Generation Sequencing (NGS) of Haemorrhagic Enteritis Virus (HEV) obtained from spleens of turkeys raised in Western Canada

Victor A. Palomino-Tapia[a], Darko Mitevski[b], Tom Inglis[c], Frank van der Meer[a], Mohamed F. Abdul-Careem[a],*

[a]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N4N1, Canada
[b]Poultry Health Services, Airdrie, AB T4A2G8, Canada
[c]The Institute of Applied Poultry Technologies, Airdrie, AB T4A2G1, Canada

In the last 10 years there has been a rise in clinical cases linked with immunosuppression (e.g. secondary bacterial infections, prolonged respiratory infections) from turkey farms in western Canada. Interestingly, these cases appear to suggest that HEV is involved as a primary agent. Although both apathogenic and pathogenic HEV strains share 99.9% nucleotide sequence homology, according to recent research, field pathogenic HEV may have been evolving to evade vaccine induced immunity. The present study is directed to answer one research question: 1) What is the genetic diversity of the HEV obtained from clinical cases in western Canada? To answer this question, spleens collected from clinical cases in turkeys where HEV is suspected to be primary agent, were homogenized, filtered, and ultra-centrifuged prior to DNA-extraction for NGS assessment. Whole Genome Sequences (WGS) from apathogenic, pathogenic, and those from HEV-suspected cases obtained in western Canada were compared and results will be discussed. The outcomes of the present project will help clarify field HEV challenge in western Canada and understand if the genetic diversity observed suggests a change in antigenicity in the field/challenge viruses.

Case Reports

Respiratory Disease and Bacterial Septicemia of Broilers Linked to Three Specific Breeder Flocks

Jose A. Linares[a], Travis Cigainero[a], David French[b], Phil Stayer[b], Erin Riley[b], Robin Gilbert[b]

[a]Ceva Animal Health [b]Sanderson Farms, Inc. Laurel, MS

Respiratory disease of broilers with lesions resembling Chronic Respiratory Disease was causing problems in broilers from two neighboring divisions in Northeast Texas. The disease condition started with normal mortality for the first two weeks, but birds were noted at that age to separate out by size. As birds matured, mortality increased with signs of perihepatitis, pericarditis, and severe airsaculitis. This case report will walk through the case in chronological order discussing epidemiological concerns, diagnostic tests, test results, treatment, tentative diagnosis, and resolution.

Respiratory Disease and Bacterial Septicemia of Broilers Linked to Three Specific Breeder Flocks

David French[a], Phil Stayer[a], Erin Riley[a], Robin Gilbert[a]

[a]Sanderson Farms, Inc. Laurel, MS

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Itch alert! There *mite* be a problem

Robinette Gilbert[a], Amy Murillo[b], J. David French[a], Phil Stayer[a], Erin Riley[a]

[a]Sanderson Farms Inc., 127 Flynt Road, Laurel, MS 39443, USA. b UCR Chancellor's Postdoctoral Fellow, Department of Entomology, University of California, Riverside, CA 92521, USA

The northern fowl mite, Ornithonyssus sylviarum and the red mite, Dermanyssus gallinae are known mite species to infest poultry (specifically broiler breeder farms). This case report describes the complications in diagnosis and control when a mite infestation occurs in a pullet farm with an unknown mite species, *Androlaelaps casalis.*

I need you to call me now!

Eric A. Heskett[a], Allen Hypes[a]

[a]Case Farms

A sudden, significant increase in mortality was reported in a flock of 57-week old broiler breeders. There were no apparent prodromal clinical signs, including drops in water and feed consumption nor declines in egg production. The only abnormality noted was a fourteen-fold increase in mortality in 24 hours. Mortality affected females and males. These findings were only reported in one house on the farm. Rapid laboratory submission and testing of fresh birds and a sample of feed ensued. Gross necropsy revealed: purple to black coloration of the tips of combs, cyanosis of the head, comb, and skin (5/7 birds), 2 of 7 birds were on feed, liver of 3 of 7 birds showed multiple necrotic foci in the parenchyma, moderate to marked splenomegaly was noted (7/7 birds), 4 of 7 birds were in production and 3 of 7 birds showed follicular regression, congestion of the kidneys were noted (7/7 birds). No gross lesions were observed in brains, hearts, airsacs, lungs or joints. Results of the feed analysis, PCR, histology, bacteriology and resolution of the flock will be presented.

Are those necks really crooked?

Danny L. Magee

Clinical Professor and Director, Poultry Research and Diagnostic Laboratory, Mississippi State University College of Veterinary Medicine

Crooked necks in breeder males of meat-type chickens have been observed for at least several years. The necks consistently crook to the birds' right side. The age when these males become apparent within the flock has commonly been after ten weeks. The incidence within the typical flock has generally been estimated to be something less than 3%. However, on rare occasion the incidence has been greater, with one flock estimated to have reached as high as 25%. With an interest in determining the cause of this condition, ten males from this highly affected flock were obtained just prior to the flock's movement to the breeder house. These males were held in a floor-pen facility for further observation and testing by neurologists, radiologists, pathologists and histopathologists. Results of these observations and related tests will be discussed.

Implications of Campylobacter Hepaticus in Organic Laying Facilities

Lisa Tenny

Kansas State University

Campylobacter hepaticus, also known as "spotty liver disease" was first reported in the 1950's and has previously only been seen sporadically, but recently is emerging in parts of Europe and Australia. Two houses on an organic commercial layer facility in the Midwest region of the United States presented with depressed birds, >1% weekly mortality, and >1% egg production losses. Gross necropsy showed severe, multifocal, white foci on the liver of all depressed birds assessed. These clinical signs and gross necropsy findings are the classic presentation for Campylobacter hepaticus. Although historically campylobacter hepaticus is difficult to culture and isolate: fresh heart, spleen, liver, and bile were collected on-farm and sent to Iowa State University for isolation of Campylobacter hepaticus. Campylobacter hepaticus was isolated and identified from the fresh bile sample submitted. Because the flock in question needed to maintain its organic status, oregano was used to improve the morbidity in the flock. This presentation will also outline: some basics on campylobacter and why it can be challenging to isolate and control, the potential sources of infection, how we think the diseases is spread, ways producers can try to prevent the disease from occurring on their facilities, information on current vaccine trials, treatment options available, and the potential for zoonosis.

Multiple Barns with High Mortality in a Large Multi-Age Layer Complex

Mohamed El-Gazzar and Yuko Sato

Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.

In two separate occasions there were significant sharp increased mortality in 3 different barns out of 7 barn multi-age layer complex. This sharp increase in mortality peaked in 5 – 7 days and declined back to normal in 3 – 4 days aided by the use of antimicrobial treatment. Birds suffered from sudden death with no apparent clinical signs. Mortality presented with blue faces and parts of combs and wattles necrotic and soft in texture. In a few birds, there were petechial hemorrhages on the tips of the periventricular glands. Others had congested/hemorrhagic lungs and ovaries. No significant drops in egg production or egg weight were reported. Samples were collected and diagnostic tests including serology, bacteriology, molecular diagnostics as well as histopathology were performed. However, no clear primary pathogen was identified as the cause of such increased mortality. This case remains with no definitive diagnosis; however, based on multiple diagnostic test results *Staphylococcus aureus* (SA) seem to be a major contributor to such high mortality. *Staphylococcus aureus* is normally and ubiquitously found in poultry and their environments. SA has been implicated in multiple

clinical presentations including most commonly arthritis, osteomyelitis and ompahlitis. However, other conditions have been reported including septicemia causing acute deaths in laying hens. This presentation will show poultry clinicians and diagnosticians a set of clinical presentations that would suggest adding SA as a cause of acute death of laying hens to their rule-out lists.

Analysis of three clinical cases related to ionophore toxicity in broiler chickens

Martha Pulido-Landinez[a]

[a]Poultry Research and Diagnostic Laboratory, Department of Population Animal Health, College of Veterinary Medicine, Mississippi State University. Pearl, MS. 39208.

Although Ionophore toxicity in broilers is not frequent, the consequences can be catastrophic, resulting in high mortality, bad productive performance. In most cases, there are no specific signs or lesions that suggest including this toxicity as important rule out in the differential diagnosis. Three clinical cases were analyzed including history and clinical signs characterized by high and acute mortality, severe respiratory distress, whitish and watery diarrhea, leg weakness, birds resting on hocks and/or reluctant to move. CK and SGT levels in blood were analyzed, these results along with microscopic examination of heart and striated muscle suggested ionophore toxicity. In two cases, ionophore levels in the feed were evaluated. In another case, biological assay was performed. Although, the first rule out in these three cases was ionophore toxicity, to get a final and conclusive diagnostic was difficult because of the variability of lesions. Results obtained for CK and SGT enzymes suggested severe muscle damage.

Three Times a Charm? Three Different Clinical Presentations of Calcium Toxicity in Meat Type Chickens.

Philip Stayer

Sanderson Farms

Clinical presentations and underlying causes were different for three different episodes of calcium toxicity documented in commercial meat type chickens. One case of calcium toxicity occurred in replacement breeding stock, first recognized when mature hens died excessively after water source vitamin D administered for eggshell purposes. Gross lesions included visceral gout in dead hens and atrophic kidneys in immature pullets. Source of calcium toxicity was due to excessive calcium formulated for pullet feed. A second case of calcium toxicity presented as elevated broiler mortality with ascites. Large particle calcium was erroneously loaded into soybean bin at the source feedmill and residual calcium particles were mixed into several batches of broiler starter feed. Replacement pullets that were reluctant to move was the initial observation with the third case. Upon physical examination, clinical phosphorus deficient rickets was diagnosed. Large particle calcium found in affected birds' gizzards proved inappropriate calcium type was incorporated into immature chicken rations. These three cases document three different manifestations and causes of calcium toxicity in commercial meat type chickens.

Feed Refusal Leading to Spiking Mortality Syndrome in Broiler Chickens

Marie K. Severyn[a], Daniel A. Bautista[b], Erin M. Brannick[c], Mark A. Dekich[a]

[a]Amick Farms, LLC, Delmar, DE, USA
[b]University of Delaware, Lasher Laboratory, Georgetown, DE, USA
[c]University of Delaware, Department of Animal and Food Sciences, Newark, DE, USA

A poultry company experienced multiple cases of lethargic broiler chickens with significant increases in daily mortality, along with some neurologic signs

on farms with two-to-three-week-old broilers. Overall, birds lacked clear gross or microscopic pathologic lesions, besides litter eating. A diagnosis of hypoglycemia was reached for these cases, often called "spiking mortality syndrome". Multiple factors influence this condition, causing acute and marked increases in daily mortality of young birds, including inadequate brooding and feed presentation, feed ingredients such as animal by-products, and mycotoxins. For these multiple cases, feed refusal from an ingredient is theorized to be the most significant contributing factor to the incidence of spiking mortality. Poultry veterinarians should be attune to nutritional influences on birds, as well as practical treatment options for managing spiking mortality cases.

Egg Drop Syndrome (Adenovirus 127) in Layers

Sherrill Davison[a], George Boggan[b], Laura Schuelkens[c], Mia Kim Torchetti[d], Mary Lea Killian[d], and Eric Willingham[e]

[a]Associate Professor of Avian Medicine and Pathology, University of Pennsylvania, School of Veterinary Medicine, 382 West Street Road, Kennett Square, PA 19348.
[b]Technical Services Veterinarian, CEVA – Biommune, 8906 Rosehill Road, Lenexa, KS 66215
[c]Colorado State University, College of Veterinary Medicine and Biological Sciences, 115 East Manor Drive Lititz, PA 17543
[d]National Veterinary Services Laboratories, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa, USA
[e]Winfield Veterinary Consulting, Inc., 1730 S. Federal Hwy, Delray Beach, FL 33483

Cage-free (3 flocks), colony cage (4 flocks) and organic (5 flocks) brown layers on two farms have been diagnosed with Egg Drop Syndrome (EDS). The etiology of EDS is a double-stranded DNA virus in the genus *Atadenovirus* of the Adenoviridae family and was first described in Holland in 1976. The natural hosts are ducks and geese and the virus is considered endemic in wild waterfowl. Potential sources of the virus include vertical transmission from breeders, contact with waterfowl, biosecurity lapses or contaminated water, egg packing supplies, vaccination supplies or needles. The diagnosis in the affected flocks was based on clinical signs and hemagglutination inhibition (HI) serological testing. A developmental molecular assay for duck adenovirus A is being investigated for diagnosis of EDS. Surveillance using HI testing of over 30 additional flocks have been negative for EDS virus. Production drops ranged between approximately 23% – 45% while some of the young flocks just coming into production did not reach peak production. The eggs were pale-shelled, soft-shelled or shell-less. In addition to the drop in production, there was evidence of a diarrhea. Mortality and water consumption were within normal limits, but feed consumption had decreased. There were minimal lesions found on necropsy examination. A watery, mucoid intestinal content was the most prominent lesion. Overall, the spread throughout these flocks was slow and continued over several weeks. The source of the virus in these flocks is unknown.

The Saga Continues: Broiler Variant Reovirus in Eastern North Carolina. Why Did it Happen?

Erin Riley, Phil Stayer, David French, Robin Gilbert

Sanderson Farms Inc., 127 Flynt Rd., Laurel, MS

Clinical cases of Viral Arthritis occurred in newer broiler production facilities in eastern North Carolina. This is despite having vaccinated the parent stock with a previously successful inactivated autogenous reovirus product produced from isolates of the larger corporate flock. The presentation will describe the problem, explore probable causes the disease took place and offer solutions. Diagnostics, epidemiology and practical aspects of vaccination will be discussed.

Chicks exhibiting drunken behavior

Lara Lamoureux, Jean-Pierre Vaillancourt, Daniel Venne

University of Montreal

In October of 2018, a Quebec chicken grower expressed concerns about chicks that had just been delivered to his farm. Birds were staggering around and were not eating properly. Chicks weighed only 70 g on day 5, which represented only about 74% of expected weight at that age. Mortality was not an issue with only six dead out of 6000 by day 4. A post-mortem of these birds showed some minor hepatic lesions and sub-cutaneous oedema of the brain area. Aggregates of macrophages and eosinophils were observed in the liver. The most significant lesions were in the brain with the degenerescence of Purkinje cells. An investigation of the feed demonstrated that these chicks were fed 2.28 Kg/tonne of the anticoccidial zoalene, instead of the recommended 0.5 Kg/tonne (0.0125%). If presented in a talk, this case would also show biochemical blood results as well as videos of the observed clinical signs.

An Outbreak of very virulent Infectious Bursal Disease Associated with Severe Mortality in 9-week-old Pullets in California.

H. L. Shivaprasad[a], R. Moeller[a], K. Takeshita[a], D. Goldsmith[a], B. Crossley[c] and S. Stoute[d]

California Animal Health and Food Safety Laboratory System –[a]Tulare, [d]Turlock and [c]Davis branches, University of California, Davis

Very Virulent Infectious Bursal Disease (vvIBD) was reported for the first time in the USA from California in 2008 in 11 and 14-week-old commercial brown pullets with a mortality of 26 % and 34 % respectively. Since then the disease has been controlled with vaccination except for sporadic outbreaks. In 2018 there was an outbreak of vvIBD in 9-week-old pullets who were not vaccinated for IBD that resulted in a mortality of 20 % in a span of 4-6 days in a flock of 70,000 birds. Clinical signs in birds included anorexia, depression, reluctance to move and sudden death. Postmortem examination of 14 birds revealed dehydration, enlarged bursa of Fabricius some of which had petechiae, hemorrhages or increased mucus. vvIBDV was confirmed by PCR on the bursa and virus isolation and sequencing. Histopathology of bursa revealed severe necrosis of lymphocytes with hemorrhages and immunohistochemistry for IBDV revealed abundant virus in the lymphocytes.

Clinical investigation of false laying syndrome in commercial egg layers

Corine Giroux

Hickman's Family Farms, Buckeye, AZ 85326

False laying syndrome is a clinical syndrome caused by infectious bronchitis virus variants in commercial laying hens, and has made a resurgence in North America in the last two years. A case, and subsequent investigation of, false laying syndrome in 1.2 million commercial laying hens the United States is described. Commercial flocks peaked at 85% production, but mortality remained within normal limits. Underdeveloped oviducts, large left ovarian cysts, yolks dropped into the coelomic cavity, and bright yellow fat due to yolk absorption were seen on gross necropsy of adult commercial egg layers in the field. Vaccination at the hatchery with a live bronchitis virus for all commercial flocks was initiated, and clinically the syndrome has since disappeared. Simultaneously, unvaccinated day old chicks were placed into the pullet house with normal flocks. These chicks were sentinel birds for isolation of bronchitis variants. At seven and ten days of age, the sentinel pullets were euthanized; and tracheas, kidneys, and cecal tonsils were shipped overnight to a diagnostic lab for virus isolation. Three bronchitis virus variants were isolated from the chicks. A molecular characterization, load and tropism analysis, and clinical infection trial is currently being performed at the University of California, Davis and those results will be discussed in a subsequent presentation.

Case study - Investigation of Aspergillosis related mortality in Commercial Layer Pullets Diagnose Rare Things Rarely

Albert M Payne, DVM, MAM, Dip ACPV

1010 Consulting Group, Inc., Ridgeland, SC

A nine-week-old flock of Bovan brown leghorn pullets presented with increased mortality that began around seven weeks of age. Both fresh mortality and clinically affected birds were submitted to the state diagnostic laboratory. Lab findings supported a diagnosis of fungal airsacculitis and pneumonia due to infection with Aspergillus spp. This paper will review the events that led to this disease outbreak and the not so unique circumstances that resulted in a brooder pneumonia syndrome at 7 weeks of age. A review of the epidemiological approach will highlight the importance of not overlooking the obvious in a disease outbreak.

Botulism in Turkeys, Is It More Common Than We Realize?

Kabel Robbins

Butterball, LLC, 307 Dodgen Place, Ozark, AR 72949

Case presentation covering several cases of botulism in commercial turkeys. Cases will represent turkey flocks of various ages, from one week to market age, clinically affected by *Clostridium botulinum*. It will raise awareness of this disease and show it may be going undiagnosed by veterinarians not familiar with its various presentations. Veterinarians will become more familiar with the disease and the need for adding it as a rule out for cases of increased mortality, weakness, and paralysis.

A Case Report of Rotavirus A Hepatitis in California Pigeons

Simone Stoute[a], Beate Crossley[b], Arthur Bickford[a], Mark Bland[c], H. L. Shivaprasad[d], Dayna Goldsmith[d], and Julia Blakey[a].

California Animal Health & Food Safety Laboratory System, University of California- Davis, CA [a]Turlock Branch, [b]Davis Branch, and [d]Tulare Branch. [c]Cutler and Associates International, Napa, CA.

The California Animal Health and Food Safety Laboratory- Turlock branch received 6 submissions of racing pigeons and squab from April to November 2018. Submissions contained live and dead adult pigeons, with owners reporting increased mortality ranging from 5 to 40%, vomiting, and diarrhea. Submitted pigeons demonstrated labored breathing and severe weakness ante-mortem. At necropsy, enlarged, congested, and mottled livers were observed. Enlarged and mottled spleens and enlarged, pale kidneys were also noted. Microscopically, lesions of mild to severe necrosis and degeneration of hepatocytes were observed, with mild to severe bile duct hyperplasia, mononuclear inflammation, sinusoidal congestion, and hemosiderosis. Mild mononuclear inflammatory cell infiltration of the pancreas and kidney was noted, with variable kidney tubular dilation and degeneration. Direct electron microscopy identified reovirus-like particles from liver sections. Reovirus-like particles were variably identified from intestinal pools by direct electron microscopy. Sequencing performed on liver samples yielded 95-98% homology over 11 genomic segments to a rotavirus A isolate from a Chinese rock dove, and 93-96% homology to a rotavirus A isolate from an Australian pigeon. A retrospective analysis determining the prevalence of reovirus-like hepatitis in California pigeons from 2000 to 2018 is also discussed. Hepatitis in pigeons caused by a reovirus-like virus has been described infrequently, has recently been characterized as a rotavirus A by genomic sequencing, and is a significant disease for the pigeon industry due to high mortality.

Vaccinology

New field experiences with dual-construct vaccines

Elise A. Myers[a]

[a]Merck Animal Health, 2 Giralda Farms, Madison, NJ 07940

Dual-construct HVT vaccines have been in use for almost two years and patterns are beginning to emerge concerning their impact on performance and potential for return on investment. In addition, advancements in technology have generated renewed interest surrounding the impact of single or dual-construct vaccines on field virus populations and in our ability to evaluate vaccine coverage and take. The impact of dual construct vaccine use in the field worldwide are discussed using a combination of field post mortem results and the evaluation of key performance indicators. In addition, any patterns detected using next generation sequencing of field virus population after use of dual-construct vaccines are presented.

Recombinant HVT/IBD Vaccine As The Sole IBDV Primer in Broiler Breeders: Seroconversion And Progeny Protection Against Challenge With Variant E

Enrique Montiel

Boehringer Ingelheim

Broiler breeder pullets were vaccinated *in ovo* with a recombinant HVT+IBDV vaccine (VAXXITEK®) + SB1 or HVT+SB1 to serve as IBDV-negative controls. The chicks were divided in three feeding groups: 1) Skip-a-day (SAD); 2) every day in the feeder (EDF) or 3) every day on the litter (EDL) and changed to daily feeding at 21 weeks of age. The IBDV-primed pullets also received an inactivated IBDV vaccine (Bursa Guard Reo, Boehringer-Ingelheim) at 20 weeks. At 4, 9, 14, 26, 30, 35, 39, 45 and 59 weeks of age, 30 serum samples were collected from each group. When the breeders were 38 and 48 weeks of age, eighty day-old broilers from each hen group were divided in 4 groups of 20 chicks each. All chicks were placed in isolation units and challenged at 14 days of age via ocular drop with IBDV Variant E, (AviServe LLC., Newark, DE). The fourth group was kept as a negative control. Protection was assessed by calculating the bursa to body weight ratio seven days after challenge. Antibody titers against IBDV in the hens at all ages tested age were very similar across feeding groups when using the same ELISA kit. IBDV antibody titers were significantly lower for kit 1 when compared with kit 2 results. Protection against challenge with IBDV variant E was not statistically different among groups. Results suggest this is an effective IBD vaccination program for broiler breeder pullets reared under various feeding programs.

Traditional live HVT and NDV -vectored vaccines protection against California 2018 virulent Newcastle Disease Virus in pullets

Alexandra Mendoza-Reilley[F], David L. SuarezA, Annette Jones[B], Pamela Hullinger[C], Rodrigo A Gallardo[D], Mia Torchetti[E], Alexandra Mendoza-Reilley[F] and Ivan Alvarado[F]

Southeast Poultry Research Laboratory[A], California Department of Food and Agriculture (CDFA)[B], California Animal Health and Food Safety Laboratory (CAHFS)[C], University of California Davis School of Veterinary Medicine[D], US Department of Agriculture (USDA) and Merck Animal Health[F].

The recent outbreak of virulent Newcastle disease virus (NDV) in California has affected at least 185 premises, primarily backyard premises. The California NDV is a genotype Vb virus most closely associated with virulent NDV viruses from Central America. Many of the affected backyard flocks were not vaccinated and the infection resulted in high mortality. Newcastle Disease outbreaks increase the awareness of how vaccines can be used to control and prevent the introduction of this foreign disease into commercial and backyard poultry premises. Viral vectored vaccines, including herpesvirus of turkeys (HVT) have become available in the United States. These vaccines allow for in-ovo or day of age application, which provides an opportunity for earlier protection from challenge. A vaccine trial

was performed with SPF white leghorn chickens to evaluate how well HVT-viral vectored vaccines that express antigens to NDV and infectious bursal disease (IBD) provide protection in combination with and without traditional live attenuated (NDV C2 Strain) vaccines. The birds were vaccinated either once with HVT-vectored vaccine or C2 at day of age and boostered with C2 virus and challenged at 20 and 28 days of age, respectively. The challenge virus was the California 2018 virulent NDV strain. Clinical disease, mortality and virus shedding at 2, 4, 7 days after challenge were investigated. The antibody response was measured using both, Hemagglutination inhibition (HI) and commercial ELISA tests. The studies will provide an important analysis of vaccination with next generation vaccines for the improved control of virulent NDV in the United States.

Compatibility of A Recominant HVT-ND Vaccine with Bursaplex to Provide Protections against Velogenic NDV, Virulent Classic IBDV and Virulent MDV Challenges in SPF Birds

Kelly Turner-Alston, Lauren Taylor, Audrey Porter, John Dickson, Jennifer Embrey, Sing Rong

Zoetis Vet Med R&D, 333 Portage Road, Kalamazoo, MI 49007

Combination of a recombinant HVT vectored ND vaccine with an immunecomplex IBD vaccine (Bursaplex) will provide protections against, Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; Marek's disease (MD), a common cause of condemnations and immune suppression in broilers; and infectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens. In this study using SPF leghorns, HVT-ND was combined with Bursaplex, and the two vaccines were either inoculated *in ovo* in E18 eggs, or injected subcutaneously on day of hatch. ND efficacy was tested against a velogenic NDV challenge at Day 28. IBD efficacy was tested against a classic virulent IBDV challenge at Day 34. MD efficacy was tested against a virulent MDV challenge at Day 5. The serology of both IBD and ND was also analyzed. The details of experimental design and study results will be presented.

The Feasibility of the IBDV MB-1 Live Vaccine Strain for *In-Ovo* and Day of Hatch Applications

Ehud Ashash[a], Caroline Noach[a], Beny Perelman[a], Charles Costello[a], Pablo Sansalone[a], Thiago Brazil[a] and Ziv Ravivb

[a]Phibro Animal Health Corporation, 2 Ha'Negev st., Airport City, 6101001 Israel. [b]ZER Veterinary Consulting, Ltd., Yehuda Hanasi 79, Herzelia, 4600826 Israel

Infectious bursal disease (IBD) is an economically important disease of young chickens caused by an Avibirnavirus, the infectious bursal disease virus (IBDV). The causative virus is highly resilient in poultry environments and vaccination is the most effective measure for IBDV control. However the suspected neutralization of maternal antibodies by highly attenuated strains and the assumed virulence of partly attenuated strains, have limited the implementation of conventional live IBDV vaccine strains in pre and post hatch chicks. Nevertheless, preliminary data has raised questions about the validity of this prevailing dogma. To investigate the possible application of a live IBDV vaccine strain, the IBDV MB-1, In-OVO to 18.5d chicken embryos and day of hatch chicks, 4 large scale global field trials have been conducted. The 4 trials have measured the relative safety, IBD immune parameters and production performances of MB-1 versus current IBDV live and immune complex vaccines in a variety of commercial broiler systems. The overall health and production performances in all 4 trials have been similar or better in the MB-1 groups.

The study's findings challenge the notion that partially attenuated IBDV strains may break through maternal immunity and induce permanent damage to the young chicks' immune response, and propose a live attenuated IBDV strain as a safe and feasible alternative for pre and post hatch broiler chicks' active immunization.

Comparison of Injection Sites for Se Bacterin Vaccine in Commercial Pullets and Layers

Katharine L. Schlist, DVM

Director of Animal Health & Welfare, Forsman Farms, Inc., Howard Lake, MN, 55345, USA

As commercial pullet rearing transitions from conventional housing to aviary-styles, methods of production must be similarly adjusted to assure pullets the best chance of success. Commercial egg-laying pullets are commonly vaccinated against *Salmonella enteritidis (Se)* including an intramuscular injection of *Se* bacterin. Four injection sites are observed for differences in physiologic and immune response between pullet groups injected with *Se* bacterin vaccine in the muscles of the medial forelimb, the pectus, the caudal hind limb, and the space within the inguinal fold. 12-week-old commercial pullets from a conventional, belted barn were selected. Pullets were housed in three adjacent columns of cages of three tiers, each cage housing 11-15 pullets. Pullets were vaccinated on the same day by the same administrator. Starting on the day of the vaccine, pullets were monitored weekly for changes in weight and injection site inflammatory reaction. Blood samples were collected at 17, 30, and 60 weeks of age for analysis of antibody titers. At 63 weeks, 5 individuals from each group were selected for post-mortem exam for grossly observable changes in the injected tissue. Results of gross live examination suggest differences in localized inflammatory response and weight change among the sample groups in the weeks following injection. Antibody titers were of similar patterns between groups. 63-week gross tissue exam results were similar between groups.

Antimicrobial

Quantifying Antimicrobial Use in Poultry Production

Randall Singer

University of Minnesota

With the need to better understand the ways in which antimicrobials are used in human and veterinary medicine, antibiotic use data collection efforts are now underway in different agricultural commodities. The objective of this continuing project is to estimate the quantities of different antimicrobials used in poultry production (broiler chicken and turkey) for specific indications and applications. For the past several years, we have been collecting antibiotic use data from poultry production companies. Data collection and analysis has now been completed for the 2013 to 2017 period. The collected data represent greater than 80% and 70% of annual U.S. broiler chicken and turkey production, respectively. Over the five-year period for which during data were collected, overall antibiotic use within the poultry industry declined dramatically. Some of this change appears to be related to the implementation of FDA GFI #213 and to changes to the VFD. Specific trends in antibiotic use practices within the broiler and turkey industries will be presented. Sustainability of antibiotic use data collection efforts will also be discussed.

Organic acids and nature identical compounds improve the efficacy of conventional antibiotics against *E. cecorum*

Benedetta Tugnoli[a], Giulia Giovagnoni[b], Philip Maynard[c], Andrea Pivab[c], Ester Grillib[c]

[a]Vetagro S.p.A., Reggio Emilia, Italy.
[b]DIMEVET, University of Bologna, Bologna, Italy.
[c]Vetagro Inc., Chicago, IL, USA

Aim of this study was to evaluate whether non-antibiotic feed additives, i.e. organic acids (OA) and nature identical compounds (NIC), can improve the

efficacy of conventional antibiotics against *E. cecorum*, responsible for bacterial chondronecrosis and osteomyelitis in poultry. For this purpose, antimicrobial susceptibility of 10 *E. cecorum* isolates was tested with micro-dilution method in presence of lincomycin, tylosin, neomycin, OA (citric, sorbic, benzoic, dodecanoic), NIC (thymol, carvacrol), or combinations of antibiotics and OA or NIC. All the strains were resistant to neomycin (up to 64 mg/L); 6 out of 10 strains were resistant to both tylosin and lincomycin (up to 64 mg/L), while the remaining strains were sensitive to both antibiotics. OA and NIC showed a strong antimicrobial activity against all the strains, with minimal inhibitory concentration (MIC) values for citric, sorbic, benzoic, dodecanoic, thymol, and carvacrol being 12.5, 100, 100, 0.12, 1.87 and 1.87 mM, respectively. Against highly resistant strains, neomycin efficacy was increased by the combination with low doses of dodecanoic, sorbic, benzoic (25% of each MIC), citric, thymol, carvacrol (50% of each MIC). Moreover, citric (50% of the MIC) increased the efficacy of tylosin (2 out of 6 strains) and lincomycin (1 out of 6 strains). In conclusion, OA and NIC were highly effective in inhibiting *E. cecorum* strains resistant to antibiotics. Furthermore, OA and NIC, when added to antibiotics, increased the efficacy of the latter, thus suggesting a possible application of OA and NIC as alternatives or adjuvants to conventional antibiotics for *E. cecorum*.

Antimicrobial effects of lactic acid and bifidobacteria probiotic strains and an enhanced organic acid product against *Salmonella*

Chasity Pender[1], Shelby Curry[1], Revathi Shanmugasundaram[2], Mohamad Mortada[2], G. Raj Murugesan[1], Ramesh Selvaraj[2]

[1]Biomin America, Inc., Overland Park, KS, U.S.A.
[2]Department of Poultry Science, University of Georgia, Athens, GA, U.S.A.

Over recent years, the topic of food safety has established itself at the forefront of media outlets, research, and government regulations. As producers shift away from antibiotic usage, industry leaders and researchers are searching for tools to manage food safety risks. Probiotics and organic acids are considered potential alternatives as they have shown to limit growth of several bacterial pathogens. An in vitro experiment was conducted to determine the antimicrobial effects of poultry-derived lactic acid and bifidobacteria probiotic strains and an enhanced organic acid product on *Salmonella* spp. The probiotic strains included *Lactobacillus reuteri*, *L. salivarius*, *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici*. The enhanced organic acid product contained a combination of sodium formate, cinnamaldhyde, and Biomin® Permeabilizing Complex (Biotronic® PX Top3US; BPX). For each of the probiotic strains, cultures were grown overnight and cell-free supernatants were collected. *Salmonella* Enteritidis was incubated overnight with four supernatant dilutions (probiotic:pathogen at 0:1, 1:1; 5:1, and 10:1) in triplicate and absorbance was measured. At 12 hours of incubation with S. Enteritidis, the 0:1 dilution for each strain had OD values ranging from 0.064 to 0.187; however, at 10:1 dilution, OD values were <0.006. For analysis of BPX, *S*. Enteritidis and *S*. Heidelberg were grown overnight with concentrations of BPX varying from 0 to 5% and absorbance was measured. Growth of *S*. Enteritidis and *S*. Heidelberg was inhibited at BPX concentrations above 0.63%. These results suggest that the evaluated probiotic strains and BPX could be viable candidates to help manage *Salmonella* populations in poultry.

Evaluation of Dry Hydrogen Peroxide for Reducing Microbial Load in a Commercial Hatchery

Brian Jordan[a], Laura McClear[a], Julia McElreath[a], Miguel Barrios[b], Jeanna Wilson[a]

[a]Department of Poultry Science, The University of Georgia, Athens, GA 30602.
[b]Synexis Biodefense, Kansas City, MO 64112

Naturally occurring bacteria in a commercial hatchery can be detrimental to hatchery performance and chick health. A method of constant sanitation could be valuable to commercial hatcheries, and a commercially available product, gaseous dry hydrogen peroxide (DHP), was evaluated for this purpose. The aim of this study was to evaluate the effects of DHP on bacteria levels in a

commercial hatchery. A hatchery with two identical sides was used, where one side was treated with DHP through the HVAC system and stand-alone units while the other half was not. Bacterial loads were measured by total ATP bioluminescence swab samples and static air plates using tryptic soy agar (TSA) taken from similar locations on each side of the hatchery for comparison. Baseline static air samples and ATP bioluminescence swabs were collected for two weeks prior to treatment. During treatment, samples were obtained bi-weekly for 27 weeks. Data analysis shows that the DHP system consistently reduced environmental bacteria in the treated egg cooler with an average 97.8 percent bacterial reduction over time. The treated setter hall showed a similar result with a 92.5 percent reduction from the beginning baseline over time. This bacterial reduction coincided with an increase in hatch of fertile eggs from the treated side compared to the non-treated, and a decrease in 3- and 7-day mortality in chicks hatched on the treated side versus the non-treated. This study suggests that DHP could be beneficial for constant sanitation to reduce microbial load and improve performance in commercial hatcheries.

Use of Formaldehyde and Hydrogen Peroxide in a Commercial Broiler Hatchery

Sue Ann Hubbard

Merck Animal Health

For decades, formaldehyde has been widely used in broiler hatcheries as a disinfectant. Even though formaldehyde has been shown to be highly effective in reducing contamination levels, it is highly toxic to humans. Exposure to high levels can be detrimental to baby chicks. Several hatcheries in more recent years have been using hydrogen peroxide to reduce contamination in place of formaldehyde. Trials measuring the efficacy of hydrogen peroxide against bacteria and fungus in a commercial broiler hatchery were conducted. Also, a synergistic effect of using both chemicals simultaneously was evaluated.

Protozoa/Parasitology

Case Study: broiler integrator performance during transition to No Antibiotics Ever production

Linnea Newman[a], Andres Montoya[a]

[a]Merck Animal Health, 2 Giralda Farms, Madison, NJ 07083

A history of performance from 2012 through July 2019 demonstrates the performance before and after transition to No Antibiotics Ever (NAE) production, which began in December 2017. The performance graphs are color coded to coccidiosis control program and are overlaid by local temperature data and coccidiosis lesion scores. Performance vs. livability, condemnation and cost spread from before to after transition are also represented. Important points: down time between flocks is the single most important factor to improve coccidiosis control. At this complex, the feed conversion improvement provided cost-justification for the longer down time. Individual cocci control programs (ionophore, vaccine-ionophore shuttle, chemical, vaccine-chemical shuttle or vaccine only) have a minor effect on cocci lesion score and performance: down time is more critical. Postmortem sessions, while only taking a very small sample of birds on a monthly basis, are demonstrated to correlate to performance. They are a useful monitoring tool, and they should be used to anticipate potential problems with dysbacteriosis or necrotic enteritis if the coccidiosis population is not under sufficient control. Based upon observations to date, an average E. maxima microscopic lesion score (Diseases of Poultry 13th Edition) of <0.50 predicts successful transition (in other words, lesion score less than +1) Livability, condemnation and cost spread give other insights into the impact of the NAE transition at this location.

Effect of litter condition (new litter vs. built-up litter) and two anticoccidial programs (bioshuttle vs. non-bioshuttle) on the oocyst concentration in feces of broiler breeder pullets

[a]Orozco, E., [b]Cummings, [b]T., Niino, T., [c]Riley, E., [c]Stayer, P and [a]Banda, A.

[a]Poultry Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University
[b]Zoetis, Inc.
[c]Sanderson Farms, Inc.

The aim of this study was to determine the effect of litter condition (new litter vs. built-up litter) and two anticoccidial programs (bioshuttle vs. non-bioshuttle) on the oocyst concentrations in feces of broiler breeder pullets. The bioshuttle (BS) program included the application of a live oocyst vaccine at the hatchery and a synthetic anticoccidial in the diet from day 10 up to 14 weeks of age. The non-bioshuttle (NBS) program only included the vaccine at the hatchery. The number of oocysts per gram (OPG) in the samples was determined by the McMaster technique. In general, the OPG values were higher, with more fluctuations in the houses with built-up litter in comparison with houses containing fresh shavings. In the houses with fresh shavings, the birds under the BS program showed a peak at the second week around 75,000 OPG, whereas the birds under the NBS program exhibited a lower peak between the 5th and 7th week with an average of 40,000 OPG. In the houses with built-up litter, a peak around 160,000 OPG was observed in the 2nd week under the BS program, and in general the OPG values were higher in comparison with the NBS program. The effects of both programs are discussed.

Anticoccidial Sensitivity Tests (ASTs): The Do's and Dont's

Hector Cervantes

Phibro Animal Health

As production of poultry without antimicrobials has continued to expand, coccidiosis prevention and control has changed. In the USA ionophore-type anticoccidials are considered antimicrobials and therefore cannot be used in programs such as RWA (raised without antibiotics) or NAE (no antibiotics ever), consequently, coccidiosis prevention and control has to be accomplished by the use of chemically-synthetized anticoccidials, the so called “chemicals”, live vaccines or combinations of the two. As with increased usage more selective pressure is placed on chemically-synthetized anticoccidials the long-term management of coccidial resistance development has become critically important. To that effect, the use of Anticoccidial Sensitivity Tests (ASTs) is a very useful tool to assess the current sensitivity of Eimeria spp. isolates from the field to the different anticoccidial drugs. When coccidial isolates representative from the field are used, and the test is properly conducted, valuable information can be collected and used to optimize the effectiveness of anticoccidial programs and minimize the risk of coccidial resistance. This presentation will review the basic concepts and benefits of ASTs and highlight the most important factors that must be considered when conducting ASTs to derive the maximum benefits.

Development of a Multi-Locus Sequence Typing (MLST) Scheme for Eimeria maxima

Ruediger Hauck, Miranda Carrisosa, Kenneth S. Macklin, Chengming Wang

Department of Poultry Science, Auburn University

Eimeria maxima is arguably the most economically important parasite in poultry production, because it is a predisposing factor for necrotic enteritis, which causes severe morbidity and mortality in broilers. Information on epidemiology and especially the re-

cycling of vaccine strains of E. maxima in the field can provide information designed to optimize the use of expensive anticoccidial feed additives and vaccines. However, a better understanding of the epidemiology of E. maxima requires a reliable method that is suited for routine application. Methods to type Eimeria spp. that have been described require cloning of PCR products or equipment that is not routinely available. The aim of the present investigation was to develop a multi-locus sequence typing (MLST) scheme for E. maxima, that can easily be applied by most laboratories. Twenty candidate genes were selected. Some genes were selected because based on MLST schemes for related Babesia spp. and Cryptosporidia hominis. The other genes were selected because homologues genes were differentially expressed between a virulent and precocious E. tenella strains. Three hundred fifty to 700 base pairs of coding as well as non-coding sequences of each gene were amplified by PCR and sequenced. Comparison of sequences obtained from commercial vaccines and field isolates with sequences obtained from GenBank showed that at least four genes showed variations and thus are suitable to be included in a MLST scheme.

Incorporating Recombinant *Eimeria* Proteins into Nanoparticles Improves Protective Efficacy Against Avian Coccidiosis

Mark C. Jenkins[a], Vjollca Konjufca[b]

[a]*Animal Parasitic Diseases Laboratory, NEA, ARS, USDA, Beltsville, MD 20705*
[b]*Department of Microbiology, Southern Illinois University*

Vaccination against avian coccidiosis by the administration of low doses of *Eimeria* oocysts is increasingly being used by the poultry industry. While coccidiosis vaccines have proven to be a reliable alternative to anticoccidial drugs, the use of vaccines necessarily introduces virulent *Eimeria* into a poultry house. Subunit antigens are an ideal alternative to live oocyst vaccines and considerable research has been devoted to its development, but so far no commercial recombinant protein-based vaccine exists. Our research has shown that orally inoculating newly-hatched broiler chicks with recombinant EmaxIMP1 protein conjugated to nanoparticles (NP) leads to localization of the antigen to various tissues and elicits a protective immune response against *E. maxima* challenge infection. This approach has been extended to the IMP1 homologue in *E. acervulina*, wherein newly-hatched broiler chicks were inoculated per os with NP-EaIMP1. Chickens immunized with NP-EaIMP1 displayed greater weight gain and feed conversion efficiency against high dose *E. acervulina* challenge compared to chickens immunized with NP conjugated to non-recombinant protein. Studies are underway to determine if NP-EaIMP1, NP-EtIMP1, and NP-EmaxIMP1 can be delivered in ovo to protect chicks against coccidiosis. Also, because only partial protection was observed relative to non-infected controls, NP-IMP1-immunized chicks will be grown in contact with *Eimeria* oocyst-contaminated litter to see if exposure to low doses of *Eimeria* can boost resistance to coccidiosis challenge early in life.

Using polymer microspheres or fluorescein to evaluate live coccidiosis delivery success following commercial coarse spray or gel-droplet administration.

Ryan P. Snyder[a], Roberto Soares[b], John R. Barta[a]

[a]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Canada*
[b]*Ceva Animal Health, Guelph, Canada*

Coccidiosis is a disease that burdens the global poultry industry causing losses in production and increased costs associated with control of the disease. One method of control is to administer live coccidiosis vaccines to day-of-age chicks at the hatchery. Most commercial broiler operations use live vaccines applied using coarse spray or gel-droplet administration. Uniform exposure of chicks to Eimeria spp. at the hatchery provides the best chance for early 'flock immunity'. Vaccine success relies on most chicks ingesting the desired dose. Directly measuring oocyst ingestion by chicks following vaccine administration (i.e. actual dose) is challenging because of the intentionally low number of oocysts in vaccines. A more easily measureable

'marker' incorporated into the vaccine could help to determine vaccine volume ingested by each chick. Two markers, polymer microspheres and fluorescein, will be used independently to measure the volume and uniformity of vaccine doses ingested by chicks after coarse spray or gel-droplet application. Boxes of chicks will be vaccinated using typical commercial applicators. After five minutes of preening, chick GI tracts from half of each treatment will be collected and processed to determine the volume of vaccine ingested following different application methods; the remaining chicks will be housed to determine total oocyst output from 5 to 7 days post-application. Infection controls will have total oocyst output determined from chicks orally inoculated with various doses of the test formulations (e.g. 0.1× to 10× doses). Total oocyst output will be correlated with ingested volumes to establish vaccine delivery efficiency of each application method.

Characterization and Control by Vaccination of a Pathogenic Eimeria Species Infecting Commercial Chukar Partridge

Jessica Rotolo, John R Barta, Ryan Snyder

The University of Guelph

A local chukar partridge (Alectoris chukar) producer has had recurrent issues with frequent clinical coccidiosis and flock mortalities as high as 15% of placed birds. Identification of the causative agent was determined to explore vaccination methods for coccidiosis management in commercial chukar partridge flocks.

To characterize this pathogenic Eimeria species, morphology, biological characterization of the life cycle and sequence-based genotyping were employed. Morphometrics of oocysts and sporocysts were measured using light microscopy with computerized image analysis. Experimental infections with coccidia free chukar partridges were used to describe the complete endogenous development and daily fecal collection post-inoculation was used to determine the prepatent period and duration of shedding. Endogenous development was determined histologically from samples collected at 8 locations along the intestinal tract every 8 hours throughout prepatency. The parasite had 5 asexual generations prior to oocyst formation over its 120 hour prepatent period; oocyst shedding persisted until 10 days post-inoculation. To complement biological data, the complete mitochondrial genome and partial nuclear 18S rDNA were sequenced. Molecular and biological observations suggest that this parasite has not been reported from partridges and will need formal description. Our understanding of the biology of this new parasite will permit testing of two vaccination methodologies to efficiently elicit protective immunity: 1) live oocyst vaccination followed by a carefully monitored 2-step partial house brooding; and, 2) a 'bioshuttle' (vaccination/anticoccidial combination). Effective coccidiosis control will enhance flock health and increase profitability for commercial chukar producers in Ontario and elsewhere.

The use of a non-ionophore anticoccidial in a bio-shuttle program for breeder pullets

Tak Niino[1], Jon Schaeffer[1]

[1]Zoetis, 1040 Swabia Court, Durham, NC 27703

Coccidiosis is one of the most important diseases in poultry worldwide. Currently, controlling coccidiosis relies on the use of vaccines, feed additives, and/or water soluble medications as part of the intervention strategy. More recently, bio-shuttle programs that incorporate a coccidiosis vaccine early followed by an ionophore coccidiostat in the feed beginning at 10-21 days of age have gained popularity in US broiler operations. The idea is to limit *Eimeria* cycling in vaccinated flocks during the period when necrotic enteritis is most likely to occur. The use of bio-shuttle programs, especially ones that use a non-ionophore anticoccidial in the feed, have not been as widely adopted for rearing breeder pullets. Most producers choose to rely on coccidiosis vaccine alone or a coccidiostat, but not both. This case explores the use of a bio-shuttle program involving a non-ionophore anticoccidial in breeder pullets.

Fifteen Years of Worming Broiler Breeder Pullets/Males

Kelli H. Jones[a]

[a]Ceva Animal Health, 8901 Rosehill Rd., Lenexa, KS 66215

Intestinal parasite control for broiler breeder pullets/males is a necessary part of poultry production in the built up litter systems of the United States. There have been many strategies for controlling intestinal parasites over the last decade or so that have shown to be successful, but there have also been many opportunities that exist, as well. When rearing pullets/males, maintaining uniformity and controlling body weight is critical to producing efficient breeders. In order to achieve this, intestinal health, including proper parasite control is crucial. In addition, worming for intestinal parasites in broiler breeders goes beyond just controlling the actual parasites themselves. Strategies for preventing diseases such as histomoniasis, are also taken into consideration when developing worming programs. This paper will cover broiler breeder worming programs, successes and failures, over the past fifteen years.

Immunology

Susceptibility and characterization of anti-viral innate immune responses in chicken B cells infected with infectious bursa diseases virus and supplemented with 1,25(OH)2D3

Juan C. Rodríguez-Lecompte[1], Jairo Jaime[2], Diana S. Vargas-Bermúdez[2], Alexander Yitbarek[3], and Julian Reyes[4].

[1]Department of Pathology and Microbiology, 4Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island,Canada,C1A 4P3.
[2]Departamento de Salud Animal, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Sede Bogotá. Colombia.
[3]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1.

Infectious Bursal Disease Virus (IBDV) targets the chicken's immune system by destroying Blymphocytes, attracting T cells, activating macrophages, and inducing a proinflammatory cytokine storm leading to cellular damage. Vitamin D plays an important role in modulating key anti-viral responses, with limited information in chickens. This study characterized the immun-virological pathways of IBDV infection and the role of vitamin D (1,25(OH)2D3) in modulating these immune response pathways in DT-40 cells, a chicken B cell line. Cells (5x105 cells/well in 24-wells plates) were cultured in triplicates followed by supplementation with 500nM of 1,25(OH)2D3 at 12h, and inoculation with 0.1 and 1 multiplicity of infections (MOIs) of a mild strain (ST-12)-live IBDV vaccine at 16h post-vitamin D supplementation. Gene expressions of antiviral markers and vitamin D receptor (VDR) were measured at 0, 3, 6, 12, 24, and 36h post infection (p.i.). Results: The earliest viral RNA was detected at 3h p.i. with a peak at 36h p.i. with both MOIs, and was independent of vitamin D. Vitamin D resulted in significant upregulation of antiviral responses (IRF7, IFN-α, OAS, PKR, viperin) and proinflammatory cytokines (IL-1β and IL-6),

albeit at different time points, and in a VDR independent manner. The key dsRNA recognizing receptor (TLR3) and the adaptor TRIF were not dependent on vitamin D. However, vitaminD did not decrease the infectious capacity of the virus or protect the cells from infection with IBDV. Our results suggest that vitamin D can have an important role in the antiviral responses against IBDV in ch-B cells. Key Words: Vitamin D, Innate, IBDV, B cells, Antiviral.

Influence of Feeding Programs on Body Weight, Egg Production and Innate Immune Responses Against E Coli and Salmonella in Broiler Breeders

Enrique Montiel,[AG] N. A. Cox,[B] R. J. Buhr,[B] J.K. Rosenberger,[C] A.J. Davis,[D] C. L. Hofacre,[E] B. M. McLendon,[F] and J.L. Wilson[D]

[A]Merial Select Inc., PO Drawer 2497, Gainesville, GA 30501
[B]USDA-ARS Poultry Microbiological Safety, Athens, GA 30605
[C]AviServe LLC, Newark Delaware
[D]University of Georgia, Poultry Science Department Building Athens, GA 30602-2772
[E]University of Georgia, Population Health Department, Athens, GA 30602-2772
[E]Pilgrims Pride, Nachitoches, LA

The impact of broiler breeder pullet feeding programs on innate immune responses was evaluated. Broiler breeder pullets were fed *ad libitum* for 2 weeks and then divided into 3 feeding groups: 1) Skip-a-day (SAD); 2) every day in the feeder (EDF); or 3) every day on the litter (EDL). All pullets were fed daily starting at 21 weeks of age. Body weights were assessed on each group at 5, 9, 13 and 17 weeks of age. Egg production was evaluated in two separate studies through 65 weeks of age in the first study and through 33 weeks of age in the second study. Innate immune response was measured by determining the SE colonization rate in spleens and ceca after oral challenge at 22, 35 and 65 weeks of age and also by the time of clearance of *E. coli* after intravenous inoculation at 17, 24 and 35 weeks of age. Body weights were significantly higher (P<0.05) in the groups fed daily. There was no statistical difference in egg production (P >0.05) between the hens fed daily or SAD in the first study. In the second study, daily hen-day egg production was significantly higher (P <0.05) in the groups fed daily. At 17 weeks of age the *E. coli* load (cfu/ml of blood) in the EDL group was significantly lower (P=0.029) than the SAD and EDF groups. *Salmonella* colonization was significantly higher (P<0.05*)* in the EDF group at 22 weeks of age.

Innate immune responses to infectious bronchitis virus in tracheal organ cultures from MHC congenic chicken lines

A.P. da Silva[a], K.A. Schat[b], H. Toro[c], R.A. Gallardo[a]

[a]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis
[b]College of Veterinary Medicine, Cornell University, Ithaca, NY
[c]Department of Pathobiology, Auburn University, AL

Through in vivo experiments, we have demonstrated that MHC haplotypes B2 and B19 are relatively resistant and susceptible respectively to IBV M41 and ArkDPI challenges. The main differences between B2 and B19 chickens were observed in humoral responses in tears at 14 days post-infection (dpi). However, cytokine measurements at 2 and 6 dpi were inconclusive. To elucidate the information gathered in our in vivo experiments, we performed an in vitro investigation using tracheal organ cultures (TOCs). Minor differences in cytokine responses were observed between uninfected and IBV M41-infected groups. Most likely the responses elicited by IBV were masked by the inflammation induced during tracheal processing. The goal of the present study is to improve the TOC technique to assess cytokine production elicited by IBV. We used a cell-adapted IBV strain to eliminate antigens present in allantoic fluid. Primary cell lines derived from B2 and B19 chickens were cultured using two different strategies: (1) rotating tubes that allowed aeration of the TOCs and (2) solid medium surrounding the TOC to keep the integrity of the tracheal lumen. Tracheas were cultured for 7 days before challenge with IBV. Rings and supernatant were collected at

12, 24, 36, 48, 60 and 72 hours post-infection (hpi). IFN-β, IL-1β, IL-6 and IL-10 mRNA levels were measured by RT-qPCR from tracheal rings and cytokine concentration was measured by ELISA from the supernatant. One ring per time point was examined by immunohistochemistry. Preliminary results will be discussed.

Establishing Reference Ranges for Blood Gas and Blood Chemistry for Commercially Grown Pullets in North Georgia using the i-STAT ® Alinity v Handheld Clinical Analyzer

Randi Clark[a], Jenny Nicholds[a], Karen Grogan[a], Steve Roney[a], Roy Berghaus[b], Daniel Venne[c]

[a]Poultry Diagnostic and Research Center, Department of Population Health, University of Georgia, Athens, GA.
[b]Food Animal and Health Management, Department of Population Health, University of Georgia, Athens, GA. cCouvoir Scott ltée, Scott, Quebec, Canada.

The number of poultry cases investigated where metabolic disturbances, rather than infectious disease, are the suspected cause of lost performance or even mortality is on the rise. Further work up on these cases has been hampered by a lack of diagnostic tests with appropriate reference intervals for interpretation. In recent years avian researchers and clinicians have improved diagnostic capabilities for understanding metabolic diseases in poultry species. Handheld clinical analyzers now have published reference ranges for poultry species. These ranges help provide a foundation for interpreting results obtained in the field, given the previous lack of appropriate reference values. The goal of this study is to provide a reference range for blood gas and blood chemistry using the i-STAT® Alinity v in immature breeders, at ten weeks of age. Three flocks from each of four companies in north Georgia were incorporated in the study. Birds sampled represent an equal number of both Ross 708 and Cobb 500 birds, two common breeds reared in the southern US. Blood samples were taken from twelve birds one hour after lights came on, both on a feed day and off-feed day. Blood analysis was performed on farm using the i-STAT® Alinity v within 10-15 minutes of collection. Data will be statistically analyzed to generate a reference interval range for commercially grown pullets at ten weeks of age. Values from feed and non-feed days will also be compared to provide insight into the fed vs fasted metabolic state of the bird.

Recombinant Avian Interferons as long lasting adjuvants in live IBV vaccines

Jose A. Cano-Buendia[1], [3]Ana Paula da Silva, Luis E. Jimenez-Martinez[1], Eduardo Guzman-Olea[2], Rodrigo A. Gallardo[3]

[1]Facultad de Medicina Veterinaria y Zootecnia, UNAM. Departamento de Microbiologia e Inmunologia. Mexico City, Mexico
[2]Consejo Nacional de Ciencia y Tecnologia CONACyT – UAEH. Hidalgo, Mexico
[3]Poultry Medicine Program, School of Veterinary Medicine, University of California, Davis

We will evaluate the use of avian interferon (AvIFN) as a genetic adjuvant. Plasmid encoding AvIFN optimized sequence under CMV promoter will be used as a nanoparticle in combination with a commercial live vaccine against avian infectious bronchitis virus (IBV). Vaccinated groups will be immunized via the oculo-nasal route at 1 and 14 days of age and challenged 7 days after the last immunization with an homologous strain. IBV specific IgG and IgA antibody titers will be measured by ELISA. Respiratory signs, viral load, tracheal histomorphometry, cilia score and quantification of AvIFN, IL-6, IL-10 and IL-1B will be analyzed. These results will help to evaluate the use of AvAIFN as a genetic adjuvant against IBV.

Evaluation of Humoral And Cellular Immune Responses Of Broiler Breeders Following Vaccination With Inactivated FadV Vaccine Adjuvanted With Emulsigen-D Or Cpg-ODN

Ashish Gupta[1], Khwaja Ashfaque Ahmed[1], Shelly Popowich[1], Kalhari Goonewardene[1], Ruwani Karunarathana[1], Davor Ojkic[1], Betty Chow-Lockerbie[1], Suresh K. Tikoo[4], Philip Willson[3] and Susantha Gomis[1]*

Inclusion body hepatitis (IBH) is an economically important fowl adenoviral (FAdV) disease of broiler chickens. Currently, there is no commercial vaccine available to control IBH in Canada. Previously, we have demonstrated the protective efficacy of inactivated live and subunit FAdV vaccines against IBH. The objective of this study was to characterize humoral and cellular responses in broiler breeders following vaccination with a FAdV-8b-SK vaccine adjuvanted with Emulsigen-D or CpG-ODN. Four groups (n=24/group) of broiler breeders were vaccinated at 16 weeks of age with FAdV-8b-SK (1x106 TCID50/bird) adjuvanted with either 20% Emulsigen-D or 50 µg CpG-ODN. Control groups were vaccinated with saline or FAdV-8b-SK with no adjuvant. Groups were boosted at 19 weeks of age with their respective vaccines. Humoral and cellular immune responses were determined by measuring serum IgY and NAb, CD4+:CD8+ T-cell ratio and the expression of IL-4 and IFN-γ in peripheral blood. Vaccine efficacy was determined by challenging broiler progeny at 14 days post-hatch. As a vaccine adjuvant, CpG-ODN induced a 0.20 to 0.30-fold higher IgY antibody response after the booster vaccination compared to Emulsigen-D. Both the Emulsigen-D and CpG-ODN adjuvanted groups induced NAb ≥2.90 log10 in broiler breeders and were equally protective (99% progeny survival, $P<0.05$) against IBH. FAdV-8b-SK vaccine adjuvated with CpG-ODN or Emulsigen-D induced IL-4 or IFN-γ positive CD4+ T-cells, respectively. CpG-ODN as an adjuvant in FAdV-8b-SK vaccine stimulated a significantly better CD8+ T-cell memory ($P<0.05$). In summary, both the adjuvants induced a durable NAb response and a mixed Th-1/Th-2 cellular response to inactivated FAdV vaccine.

Assessing the correlation between breeder flock serology and maternal antibody titers of turkey poults

Katie M. Stumvoll

Staff Veterinarian, Jennie-O Turkey Store

Maternal serology and serology of 0-1 day old poults will be evaluated to determine transfer of passive immunity in commercial turkeys. This information will be used to decide if tailoring vaccination protocols based on week of lay is warranted.

Bacteriology

Field Trial of Live Mycoplasma synoviae (MS) Vaccine in Area of High MS Prevalence

Myeong-seob Kim[a], Tae-min Lab, Seong-woo Cho[b], Sang-won Lee[b]

[1]Boehringer Ingelheim Animal health Korea, [2]Veterinary Microbiological laboratory of Konkuk University.

The prevalence of *Mycoplasma synoviae* (MS) in Korea is very high (>70%). It is expected that MS vaccinated flock will be exposed to field MS strain. The purpose of this study was to evaluate efficacy of a live MS vaccine against field strain exposure in farm condition. For evaluation, flocks vaccinated with the live MS vaccine were monitored by ELISA. Also, re-isolation and identification of the MS strain from birds was conducted using Multilocus sequence typing. The results showed an increase in serological response after vaccination and exposure of field strain in vaccinated flocks. The re-isolation results confirmed the protective efficacy of the live MS vaccine in field conditions. As an interesting case, there was an observed effect of decreased re-isolation rate of the MS vaccine strain due to intensive antibiotics use on vaccinated flock. This study presents a good example of how the live MS vaccine works in the poultry farm and what can be expected from the vaccine in area of high MS prevalence.

Development and validation of Mycoplasma iowae (MI) Core Genome Multilocus Sequence Typing (cgMLST) scheme

Mostafa Ghanem[a], David Ley[b] and Mohamed El-Gazzar[c],

[a]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA; Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA[c]; Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.

Mycoplasma iowae (MI) Infection causes a vertically transmitted disease of turkeys. It results in economic and commercial implications. Investigation of transmission dynamics and identifying infection source during outbreaks are required to facilitate the control and eradication efforts of MI. Recently, we developed MLST for typing and differentiation of MI strains. MLST revealed the relatively clonal MI population structure with all typed North American strains belonging to a single clonal complex (CC). However, to differentiate between closely related strains belonging to a single outbreak or CC, more discriminatory power is needed. Additionally, the declining cost of sequencing led to availability of more Whole Genome Sequences (WGS). However, there is no standardized method for typing MI strains based on whole genome sequences. In this study, we are proposing a core genome multilocus sequence typing (cgMLST) scheme as a potential standardized and reproducible method for typing and differentiation of MI genomes. The first complete genome for MI type strain was generated and used as reference for identification of MI cgMLST targets and development of the cgMSLT scheme. A diverse collection of MI WGS were generated and typed using this scheme. cgMLST was also used for evaluation of conventional MI MLST scheme. Moreover, the high discriminatory power of cgMLST allowed differentiation between samples of the same MLST type. cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation between MI isolates. Additionally, it provides stable and expandable nomenclature, allowing for comparing and sharing the typing results between different laboratories worldwide.

Clinical 6/85 *Mycoplasma gallisepticum* Vaccine Infection in Pre-Production Breeder Turkeys

Judith LaBounty[a]

[a]Staff Veterinarian, Rembrandt Foods, Spirit Lake, Iowa, United States.

Mycoplasma gallisepticum (MG) infection is a respiratory disease well described in the literature. A flock of pre-production parent stock breeder turkeys presented with respiratory clinical signs. Gross examination of the flock, serology and molecular diagnostics confirmed infection with MG. Genetic sequencing of the MG isolate indicated that the flock was infected with 6/85 vaccine strain MG. This case demonstrates that turkeys can become clinically infected with 6/85 vaccine strain MG.

Campylobacter and microbiome interactions in broiler litter: a matched case-control study

R. Valeris-Chacin[1], B. Wass[1], G. Moores[1], K. Danielson[1], J. Goldschmidt[1], M. Pieters[2], R. Singer[1]

[1]Dept. of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN. [2]Dept. of Veterinary Population Medicine and Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN.

Campylobacter is one of the leading causes of bacterial diarrhea in the US. Chicken meat is one of the main sources of infection. However, the role of the litter microbiome in the persistence of *Campylobacter* in broiler houses is still unclear. The objective of this research was to determine changes in the litter microbiome that are associated with an increased probability of *Campylobacter* positivity. Samples collected as part of an on-farm broiler

chicken antimicrobial resistance surveillance program (in collaboration with USDA-APHIS and FDA) were used in a matched case-control design. Each litter sample corresponded to one flock. *Campylobacter*-positive litter samples, the cases, and *Campylobacter*-negative litter samples, the controls, were matched on the broiler house. *Campylobacter* culture was used in the outcome assessment. DNA extracted from the litter wash was used for 16S rRNA gene sequencing (MiSeq platform 2 x 300 bp) in the exposure assessment. Reads were processed using Mothur. Alpha diversity (inverse Simpson's diversity index), beta diversity, and differential bacterial abundance were used as predictors of *Campylobacter* status in a series of conditional logistic regression models adjusting for age of the flock. Litter with higher alpha diversity had higher odds of being *Campylobacter* positive after adjusting for age of the flock (aOR for 100 units=1.06, 95% CI=1.002-1.12). Moreover, some differentially abundant bacteria were significantly associated with *Campylobacter* in litter after adjusting for age of the flock: *Bacteroidetes* (aOR=2.04, 95% CI=1.21-3.43), *Anaerofilum* (aOR=5.8, 95% CI=1.29-26.05), *Corynebacteriaceae* (aOR=0.14, 95% CI=0.02-0.94), and *Enterococcaceae* (aOR=0.09, 95% CI=0.01-0.78). The association of *Campylobacter* with *Lactobacillus* and *Clostridium* species in the litter was antagonistic (aOR=0.82, 95% CI= 0.29-2.37, and aOR= 0.83, 95% CI=0.68-1, respectively), although without statistical significance. These results suggest that bacterial interactions in the litter microbiome foster or hinder the survival of *Campylobacter*.

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

Dan Karunakaran, Sam Anderson, Rebecca Wujek, Eric Vang, Evan Hutchison, Jodi Delago, Alexandra Smith and Tom Rehberger.

Arm and Hammer Animal and Food Production, Waukesha, WI

Escherichia coli are ubiquitous in the environment and constitute part of the normal microbiota of healthy poultry. However, there is a wide range of genetic diversity among *E. coli* isolates and the genomes of some lineages contain several virulence factors. For example, avian pathogenic *E. coli* (APEC) comprise a specific subset of *E. coli* that cause extra intestinal diseases of poultry. Surveys of APEC lineages from broilers and turkeys over the production cycle indicate that the number and type of virulence factors change from day-of-hatch and through production. An in-depth understanding of the genetic diversity of the APEC will aid in determining the source of the pathogens and in determining their sensitivity to interventions such as probiotics aimed at reducing APEC levels in the gastrointestinal tract. Results from a four-year industry survey involving commercial broilers and turkeys will be presented.

Characterization of omphalitis causing Extra-intestinal pathogenic *Escherichia coli*

Martine Boulianne[a], Rébécca Vachon[a], Luc Verrette[a], John M. Fairbrother[b], Ghyslaine Vanier[b], and Lila Maduro[a]

[a]Chair in Poultry Research, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe (Québec), J2S 7C6, Canada.
[b]OIE Reference Laboratory for Escherichia coli (EcL), Université de Montréal, C.P. 5000, Saint-Hyacinthe (Québec), J2S 7C6, Canada.

Antibiotics at the hatchery have been used to prevent omphalitis in chicks, an infection mainly associated with *Escherichia coli*. With the current pressure to reduce antibiotic use, a better understanding of its causal agents is needed in order to find alternative control measures. Our objective was to characterize of extra-intestinal pathogenic *E. coli* strains (ExPEC) found in the yolk sac of chicks with omphalitis. Swabs from the yolk sac of dead chicks presenting lesions suggestive of omphalitis were collected from ceftiofur and lincomycin-spectinomycin hatchery treated chicks, and streaked onto a McConkey agar. Three colonies per plate were selected and after growth in LB broth, DNA extraction, isolates were confirmed to be *E. coli* by PCR for detection of the housekeeping gene uidA. In order to characterize the isolates, the presence of five virulence genes was tested by PCR; iucD, ompT, hlyF, iss and iroN. For both groups, results suggest

that a single strain of virulent ExPEC takes over with two thirds (67 and 68% respectively) of the chicks showing their three picked ExPECs isolates with a similar virulence profile. Again, for both groups, 70 and 65% of these isolates possessed all five virulent genes. The rest of the omphalitis cases showed two and three different virulence profiles per swab suggesting a mixed infection. Interestingly analysis of uidA negative bacterial strains with a MALDI-TOF showed the presence of *Citrobacter, Klebsiella*, *Acinetobacter, Salmonella*, *Pseudomonas* and *Proteus* spp. This would suggest that while omphalitis is mostly caused by a single strain of a pathogenic *E.coli*, a third of the omphalitis cases might be secondary to an opportunistic infection hence could be prevented.

The role of *Enterococcus faecalis* in the modulation of avian pathogenic *E. coli* infections of poultry

Grayson K. Walker, Mitsu Suyemoto, Siddhartha Thakur, and Luke B. Borst

Population Health and Pathobiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC

E. coli and Enterococcus spp. co-evolved as commensal microbes in the gastrointestinal tracts of many species including domestic poultry. Interestingly, these bacteria are also frequently co-isolated from young birds with colibacillosis, a fatal septicemic disease. While the role of avian pathogenic E. coli (APEC) in colibacillosis has been extensively studied, the contributions of co-isolated Enterococci to the pathogenesis of colibacillosis has largely been ignored. In this preliminary study we characterized 16 pairs of APEC and Enterococcus faecalis (EF) co-isolated from colibacillosis lesions using antimicrobial resistance phenotyping, whole genome sequencing, and a chick embryo lethality assay of virulence. Antimicrobial resistance was frequently detected in both APEC and EF isolates with tetracycline resistance commonly shared by co-isolates. However, in APEC, resistance was mediated by tetA and tetB compared to tetM and tetL in EF. Virulence genes identified in APEC isolates varied in number with 1 to 9 identified per isolate. The siderophore receptor iroN gene was found in 13 of the 16 APEC isolates. As EF has been shown to facilitate iron acquisition by E. coli, co-isolates were cultured as individual macrocolonies with increasing proximity to one another on iron-depleted medium. APEC colonies most proximal to EF macrocolonies exhibited increased growth compared to the more distal APEC macrocolonies ($P \leq 0.01$). Co-infection with APEC and EF increased mortality in the embryo lethality assay compared to APEC or EF alone. These data suggest that EF may play a significant role in modulating APEC virulence during polymicrobial infections of poultry.

Attempts to reproduce focal duodenal necrosis by experimental infection of chickens with *Clostridium perfringens* isolates and duodenal homogenates

Monique França[a], Ana M. Villegas[a,b], Lisa Stabler[a], Claire-Sophie Rimet[a], Carmen Jerry[a], Barquiesha Madison[a]

[a]Poultry Diagnostic and Research Center, Department of Population Health, University of Georgia, Athens, GA 30602.
[b]Department of Poultry Science, University of Georgia, Athens, GA 30602

Focal duodenal necrosis (FDN) is an intestinal disease of table egg layers. The objective of this study was to try to reproduce FDN by experimental infection of egg laying chickens. We performed a series of experiments using different *netB*-positive and *netB*-negative *Clostridium perfringens* isolates and duodenal homogenate obtained from FDN lesions. Distiller's dried grain with solubles (DDGS), high protein diet containing fishmeal and limestone were added to chicken feeds as potential predisposing factors for disease development in these experiments. Chickens challenged with *C. perfringens* and/or duodenal homogenate developed gross lesions characterized by mucosal hyperemia, mild mucosal erosions, mild hemorrhage, and the presence of watery and frothy contents in the lumen of the duodenum. Histopathology revealed mild to moderate lymphoplasmacytic and heterophilic inflammation in the lamina propria, hemorrhages in the lamina

propria and lumen, minimal to mild enterocyte degeneration and necrosis as well as lesions in intestinal crypts. *Clostridium perfringens* and duodenal homogenates from FDN lesions cause gross and microscopic lesions in the duodenum of experimentally infected chickens. The development of more severe FDN lesions may require the presence of other infectious agents and predisposing factors that still need to be determined.

16s Phylogenomic Analysis of Environmental and Tissue Microbiota for Correlating Management Strategies Related to Incidence of Clostridial Dermatitis in Turkeys

Britney Rauk[b], Paul Plummera,[c,d], Adam Krull[e], Ganwu Li[f], Yuko Sato[a,f]

[a]Iowa State University, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011.
[b]Iowa State University, Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA 50011.
[c]Iowa State University, Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. [d]Executive Director, National Institute of Antimicrobial Resistance Research & Education.
[e]DuPont Pioneer, Johnston, IA 50131.
[f]Iowa State University, Veterinary Diagnostic Laboratory, 1850 Christensen Drive, Ames, IA 50011.

Clostridial dermatitis of turkeys is a condition which causes mortality and plant condemnation in market age turkeys. It is a nationwide health concern and ranks as the third most important issue facing the turkey industry. The objectives of the study are to correlate the etiology of this condition with various environmental factors, as well as to analyze samples with 16S phylogenomics analysis to observe potential shifts in microbiota in diseased animals. Fecal samples, water swabs, and litter were collected weekly from 6 locations in Iowa. Three control and three case farms were utilized for sample collection. Flock managers/supervisors were asked to fill out a detailed questionnaire including ABF status, source of birds, litter management, vaccination history, history of antibiotic or anthelmintic use, use of feed additives, biosecurity measures, etc. Case flocks qualified if mortality from TC exceeded 0.5 per 1000 birds for two consecutive days. In case barns, fecal samples and select organs were collected aseptically at necropsy and submitted for bacterial determination and metagenomic analyses. External temperature, humidity, and litter temperatures were recorded weekly. Dry matter percentage and particle size analysis were performed from each litter sample. Further analysis will help determine associations of management practices correlating with trends in microbiota in the environment, healthy turkeys, and diseased birds.

Ornithobacterium rhinotracheale (ORT): Never ending story

Hafez Mohamed Hafez

Institute of Poultry Diseases, Free University Berlin, Germany

Infection with O. rhinotracheale has been recognized in many countries worldwide and incriminated as a possible additional causative agent in respiratory disease complexes in poultry. The infection in turkeys and chickens is mostly associated with heavy economic losses. Clinical signs and lesions are of little value in diagnosis, since many other infectious diseases can produce similar clinical signs and post mortem lesions. Accurate diagnosis must be substantiated by laboratory investigations. The control based mainly on biosecurity to prevent the introduction and spread. ORT has been shown to be highly sensitive to different chemical disinfectants. On the other hand, the sensitivity to antibiotics are inconstant and showing regional variation. In several place commercial or autogenous inactivated oil-adjuvant vaccines are used and able to reduce mortality and condemnation rates. The paper describes besides the history the most current challenges related to diagnosis, serotyping, pathogenicity and control measures.

In vitro and *in vivo* evaluation of probiotic supplementation and its effect on performance and immune response in *Campylobacter*-challenged broilers

Mohamad Mortada[a], Revathi Shanmugasundaram[a], G Raj Murugesan[b], Ramesh Selvaraj[a]

[a]*Department of Poultry Science, The University of Georgia, Athens, GA, USA.*
[b]*BIOMIN America Inc., Kansas City, KS, USA.*

Campylobacter is one of the major foodborne pathogens associated with the consumption of poultry. *In vitro* and *in vivo* studies were conducted to evaluate the effectiveness of *L. reuteri*, *B. animalis*, *E. faecium* and *P. acidilactici* for the reduction of *C. jejuni* in broilers. The *in vitro* study aimed at determining the inhibitory effect of probiotic supernatants against *C. jejuni*. An *in vivo* study was conducted to evaluate the effect of *C. jejuni* challenge and probiotic supplementation on performance and immune parameters in broilers. Birds were randomly assigned to 4 treatments with 6 replicates/treatment (n=6) and 8 birds/replicate. Treatments 1 and 3 received synbiotic supplementation daily in feed (0.1% wt/wt) while treatments 2 and 4 received a basal diet. Birds in treatments 1 and 4 were orally challenged with 1 X 108 CFU/bird of *C. jejuni* on d 14. *C. jejuni* challenge decreased BWG by 82g on d 35 (p=0.22). In addition, challenged birds had a significantly higher (p<0.01) CD4+CD25+ % in cecal tonsils when compared to unchallenged birds at 7 d post challenge (5.56% vs. 0.76%). Treatment 1 had decreased CD4+CD25+ % by 38.7%, 39.7% and 12.3% when compared to treatment 4 on 7, 14 and 21 d post challenge, respectively. Treatment 1 had a lower CD8+/CD4+ ratio when compared to the other treatments on d 21. In conclusion, the 4 probiotic strains showed an inhibitory effect against *C. jejuni in vitro* and were able to decrease CD4+CD25+ % and Th1/Th2 ratio in *C. jejuni*-challenged birds *in vivo*.

Diagnostics

Evaluation of sample collection devices for recovering poultry respiratory viruses from the environment

Christopher B. Stephens[a], Erica Spackman[a], Brian Jordan[b,c], and Casey Ritz[c]

[a]*US National Poultry Research Center, USDA-Agricultural Research Service, Athens, GA, 30605 USA.*
[b]*Department of Population Health, Colleges of Veterinary Medicine and Agricultural and Environmental Science, University of Georgia, Athens, GA 30605, USA.*
[c]*Department of Poultry Science, College of Agricultural and Environmental Science, University of Georgia, Athens GA 30605 USA.*

A critical step during recovery from an outbreak of a poultry respiratory virus, such as avian influenza virus or Newcastle disease virus, is environmental sampling to confirm the absence of the virus prior to releasing quarantine. Numerous sample collection devices have been used in the past, but the best device (most sensitive and cost effective) has not been identified. In order to identify the best device, four common environmental sample collection devices were evaluated: polyester swabs (similar to those used to swab birds), large tip foam swabs, cotton gauze, and cellulose sponges. A contaminated poultry premises was simulated by spray vaccinating commercial broilers and their indoor environment with a live Massachusetts serotype infectious bronchitis virus (IBV) vaccine, which was used as a surrogate for other poultry respiratory viruses. The poultry house was divided into floor pens, and a total of seven locations inside each pen, and three facility locations inside the house, but outside the pens were swabbed every twenty-four hours. Samples were collected on the day of vaccination through five days post vaccination. Recovery of IBV by each device was quantified by real-time qRT-PCR. The cotton gauze demonstrated the highest mean titers of IBV at each location swabbed and there was the highest percentage of positive samples with gauze at each

location. Other devices were less consistent for virus recovery.

Re-emerging Turkey Arthritis Reovirus - Diagnostic Strategies

Sunil K. Mor, Pawan Kumar, Maria Barrera, Sagar M. Goyal and Robert E. Porter

Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN 55108, USA

Turkey arthritis reovirus (TARV)-induced lameness in turkey flocks continues unabated. In 2017, University of Minnesota Veterinary Diagnostic Lab (MVDL) received 268 cases from 11 different states of which 169 (63%) were positive for TARV. In the first six months of 2018, we received 130 cases of which 65 (50%) were positive. Current diagnosis of the recently re-emerged turkey reoviral arthritis relies on both virus isolation and PCR. We have developed a real time RT-PCR (qPCR) for the detection of TARV and a universal avian reovirus qPCR for detection of all avian reoviruses. Veterinarians have sought diagnostic serum assays, but current commercial assays for reovirus are chicken-specific and yield unreliable results. We have developed a whole-virus ELISA to detect turkey reovirus (TRV) antibodies in infected turkeys. Our TRV ELISA is capable of detecting antibodies from different TRV strains currently circulating in the US turkey flocks. Enzyme-linked immunosorbent assay (ELISA) would be effective for monitoring antibody titers in both breeder turkeys that are vaccinated for reovirus and the offspring from those breeders. Detection of high anti-reovirus titers at a later age might allow producers to identify a field challenge, giving them time to alter the processing schedule and salvage more birds. Additionally, we have developed hyperimmune sera against 20 different isolates, which will be used in the development of cross neutralization assay. We have developed protocol for complete genome sequencing of avian reoviruses and a bioinformatics pipeline for in-depth sequence analysis. These results will be discussed in detail during the conference.

Avian Reoviruses: Molecular Characterization In California

Rodrigo A. Gallardo, Sofia Egaña-Labrin, Simone Stoute, H.L. Shivaprasad, Beate Crossley, Ha-Jung Roh

University of California, Davis

Reovirus variants induce high economic losses in the affected flocks due to lack of uniformity, poor feed conversion, increased condemnations and reduced animal welfare in meat-type poultry. In California, a reovirus tenosynovitis outbreak started in August 2015 affecting broilers from 14 to 47 days of age. We have focused in virus isolation, molecular characterization and pathogenicity of some of these isolates. Most of the recovered isolates are from tendons, followed by heart and joints. Six different genotypic clusters have been recognized with changes in their trend from 2015 to 2018. Pathogenicity studies show microscopic lesions in heart and tendons. Full genome sequencing techniques are helping us to determine associations between pathogenicity, antigenicity and certain genes.

Molecular Characterisation of Chicken Arthritis Reoviruses Circulating in Israel, Germany and the United States

Rahul Kumar, Sunil K. Mor, Pawan Kumar

University of Minnesota

Chicken arthritis reovirus (CARV) causes arthritis/tenosynovitis in chickens with or without gastrocnemius tendon rupture. Current vaccines are not effective again recently emerging U.S. variants of CARV, and there are no uniform criteria for defining variant genotypes. In the past year 47 new CARV isolates from outbreaks of tenosynovitis have been collected in Israel. Based on SI gene sequencing, 25 CARVs from Israel were classified as Genotype-2 with 0.3-0.9% difference among the 2017 and 2018 isolates. Passaging 40 different isolates in multiple cell lines showed that Vero cells were the preferred cell line in terms of rate of virus production. A total of 104 CARV isolates, 30 from

Israel, 33 from Germany, and another 35 CARVs (U.S.) and six chicken reoviruses (CRVs) associated with runting-stunting syndrome (U.S.) were processed for NexGen Sequencing. The extracted viral RNA samples were submitted to University of Minnesota Genomics Center for cDNA synthesis, library preparation and Illumina Miseq 300 paired end cycle run. We have developed a bioinformatics pipeline for analysis of NGS data for avian reoviruses. At this point, the complete genome of seven U.S. isolates have been assembled. The phylogenetic analysis of all 10 segments revealed that CRV sequences formed different groupings indicative of re-assortment. The maximum nucleotide and amino acid variations were observed in S1, S3 and M2 gene segments. The complete genome analysis of all 104 isolates will help in understanding the re-emergence and evolution of CARVs in the United States, Israel and Germany.

Developing and Application of Real time PCR protocols to Differentiate *Mycoplasma synoviae* Vaccine Strains

Mohammadreza Ehsan, Marianne Dos Santos, Amanda Olivier and Naola Ferguson-Noel

Department of Population Health, Poultry Diagnostic and Research Center, University of Georgia, Athens, GA 30602

Mycoplasma synoviae infection may result in large losses to the poultry industry that make control and prevention measures (such as vaccination) essential. For *M. synoviae,* live attenuated vaccines are the most commonly used vaccines to prevent the infections with this organism, so that differentiating the vaccine strains and field strains, which is only possible by molecular assays, is critical in choosing the right strategies and management practices. Although several molecular techniques, such as DNA sequencing of specific genomic targets (primarily *vlhA*), and multi-locus sequence typing (MLST), have been proposed for strain differentiation, a more rapid but less costly assay with similar or better specificity and sensitivity would be advantageous. In this research, strain-differentiating primers and probes for Taqman® real-time PCR were developed based on specific targets and then tested on DNA extracts from pure cultures of different strains of *M. synoviae*. The assays were also tested on DNA extracts from cultures of other mollicute *(Mycoplasma and Acholeplasma)* species to assure the specificity. Finally, the assay was tested on tracheal swabs from negative and infected (some vaccinated) chickens, as well as samples submitted for routine diagnostic testing, including FTA® cards. Application of these protocols allows rapid differentiation between *M. synoviae* strains in a quantitative manner.

Infectious Bronchitis in Immunologically Naïve Breeding Chickens and Their Progeny

Corissa A. Steimling[1], Eva A. Wallner-Pendleton[1], Tamer A. Sharafeldin[1], Huaguang Lu[1], Jennifer L. Williams[1], Patricia A. Dunn[1], Robert L. Owen[2], Meagan Slater[2], Brian Ladman[3], Ha-Jung Roh[4]

[1]The Pennsylvania State University Animal Diagnostics Laboratory, University Park, PA, USA
[2]Best Veterinary Solutions Inc., Mechanicsburg, PA, USA
[3]University of Delaware, Department of Animal and Food Sciences, Georgetown, DE, USA
[4]Ceva Biomune, Lenexa, KS, USA

In April 2018, a sudden and significant decrease in egg production was reported in a fifty-two-week-old breeder flock of specialty chickens in Pennsylvania. The flock was assumed to be immunologically naïve, since routine vaccination had not been pursued for over a decade on this farm. Both hens and eggs were submitted to the Pennsylvania State University Animal Diagnostic Laboratory for examination and testing. Diagnostic results indicated an active Infectious Bronchitis Virus infection. Tissues were sent for genotyping via rRT-PCR, and only DMV/1639/11 was detected. During the active infection, there were six different age groups of progeny from the affected breeder flock being hatched and reared within the same management system. All the progeny groups were tested, and were positive for IBV, which was also genotyped as DMV/1639/11. Ten weeks after the confirmed diagnosis of a pure DMV/1639/11 infection, birds were selected from each of the tested pullet pens for necropsy. There was an incidence of severe,

segmental cystic left oviduct in each of the groups. To the knowledge of those involved with this case, this is the first report of the effects of IBV DMV/1639/11 in immunologically naïve immature and mature chickens.

Infectious laryngotracheitis prevalence in California backyard chicken flocks and strain differentiation by ICP4 sequencing, 2007-2017.

Julia Blakey[a], Simone Stoute[a], Beate Crossley[b], Aslı Mete[b]

[a]California Animal Health & Food Safety Laboratory System, University of California - Davis, Turlock Branch, Turlock, CA.
[b]California Animal Health & Food Safety Laboratory System, University of California - Davis, Davis Branch, Davis, CA.

A retrospective investigation of infectious laryngotracheitis (ILT) infection in California backyard chicken flocks (BYF) from 2007 to 2017 was performed. Eight-eight BYF case submissions were diagnosed with ILT at the California Animal Health and Food Safety Laboratory System (CAHFS) during this time period. ILT diagnosis by year varied from 0.19% to 1.7% of the total BYF submissions, resulting in significant disease outbreaks and mortality in affected flocks. Premise location, clinical signs, macroscopic and microscopic lesions, diagnostic methods, and management options were analyzed. In order to facilitate differentiation between ILT field strains and vaccine strains, real-time polymerase chain reaction (rtPCR) analysis and sequencing of 996 base pairs (bp) of the infected-cell polypeptide 4 (ICP4) gene was performed on 15 BYF ILT samples. The resulting ICP4 sequences were compared to reference field strains and vaccine strains (LT-Blen®, Laryngo-Vac®, and LT-Ivax®) in GenBank. Nine out of 15 strains were 100% identical to LT-Blen® in the partial ICP4 sequence analyzed. Another 5 strains were 98.8% identical to the LT-Blen® vaccine strain except for a 12bp insertion. One strain was 99.9% identical to the Chinese isolate WangGang (DQ995291) in the partial ICP4 sequence analyzed. ILT outbreaks are frequently caused by escaped CEO vaccine strains, and BYFs are capable of serving as a reservoir for ILT infection of nearby commercial flocks. The presence of ILT in BYFs in counties where commercial poultry have high concentrations demonstrates the risk for disease transmission, and emphasizes the importance of continued surveillance in BYFs.

Management

Evaluation of enrichments for meat-type chickens

Kate Barger-Weathers

DVM, Director of Global Animal Welfare, Cobb-Vantress, Inc., Siloam Springs, Arkansas, USA

While there is no consistent definition for enrichments, they have been described as something that should improve the biological functioning of an animal. Enrichments are often incorporated into the environment as they reduce negative, stereotypical behaviors, and may improve the welfare outcomes due to social, occupational, physical, or sensory stimulation. Enrichments have been incorporated into the environments of zoo animals and are also used for companion animals. The use of enrichments in food animal production has not been well-researched. Nonetheless, retailers, restaurants and welfare groups are beginning to require enrichments for broilers, but the benefit of the enrichments for young, meat-type chickens is not well understood. As a result, many items have been tested to enhance the lives of indoor-reared broilers. With regards to the use of enrichments for breeders, few studies have evaluated enrichments and presently there are no mandated requirements for enrichments in breeder chicken environments. However, the use of enrichments on rearing farms has potential since they may provide these birds with environmental stimulation, and furthermore prepare the birds for the environment they will encounter on laying farms. This paper will present data from studies that evaluated various floor-based and suspended enrichments for meat-type chickens. Information will include the type of enrichment, analysis of the frequency and duration of use, welfare outcomes, and performance outcomes for the poultry flocks. This paper will also present practical suggestions for

improving welfare outcomes and biological function of broiler breeder chickens via the incorporation of specific enrichments in commercial poultry farms.

Effects of sanitation water treatments during broiler growth-out

Ricardo Munoz

Neogen

Water source uniqueness is due to the substances dissolved in it. This will determine water properties, such as taste, hardness, pH, biofilm and bacteria growth.
Materials & Methods
Broiler males Hubbard x Ross 708 were place in 72 pens, 27 birds per pen on fresh or used litter, and maintained at stocking density approaching the commercial standard to replicate production and welfare aspects that can occur in a commercial poultry house (0.75 sq ft/bird) In order to measure broiler performance by: body weight (BW), feed conversion (mFCR), water consumption, and chronic stress; by adrenal gland weight asymmetry (AGA). Feed and water ad libitum, for 35 days. Birds received either tap water (control) or water with fully activated chlorine dioxide (ClO2) and partially activated ClO2. Water treatment verification was assessed by sampling at the point of adding the sanitizer (Bin) and at the end of the water line for nipple drinkers, where the products should demonstrate the maximum reach of activity. The activity of the products was measured through pH, total chlorite and free CLO2.
Results/Discussion
Both water treatment methods could help to alleviate health response and prevent poor performance under poultry production facilities. Even though the quality of the tap water was over the regular standards from broiler houses, the results demonstrate that water treatments had different benefits for the parameters under analysis.
Conclusion
No differences in overall water intake between water treatments (P=0.461) and under below-average water quality conditions, or poor cleaning of water line drinking systems, both water treatments could demonstrate a higher return of investment benefit under field conditions

What are you doing to day-old turkeys?

Ben Wileman[a], Mark Peterson[b]

[a]Director of Veterinary and Avian Sciences, Select Genetics, 1800 Technology Dr NE, Willmar, MN.
[b]Data Scientist, Life Science Innovations, 1800 Technology Dr NE, Willmar, MN

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

Implementation and Evaluation of a Biosecurity Audit Tool Within a Large Turkey Company

Molly E. Parker[A]

[A]Butterball, LLC, 1 Butterball Lane, Garner, NC 27529

A large turkey company undertook the development and implementation of a biosecurity audit tool specific to their company practices. This biosecurity audit tool contained 27 specific criteria, and the criteria were divided into 2 broad categories: "farm risk factors" and "house risk factors." Each criteria was assigned a point value of two points or three points. Three points were assigned to criteria which were known risk factors

for a highly pathogenic avian influenza (HPAI) outbreak, as noted in the United States Department of Agriculture (USDA) Animal & Plant Health Inspection Service (APHIS) epidemiology report of the 2015 HPAI outbreaks. The audit tool allowed for 60 points total, with achievement of 51 points (85%) deemed a "passing" score. Upon implementation, this audit tool was completed by a company service technician on every farm once per quarter. One major goal of this audit tool was to provide a numerical score around a particular farm's biosecurity practices so that the company could determine which farms were at an increased risk for various diseases. This audit tool also allowed for documentation of poor biosecurity practices which helped to strengthen the company's ability to place growers on a performance improvement plan (PIP) due to lack of adequate biosecurity.

Impact of downtime on performance and livability in commercial turkey flocks

E. C. Gerken, DVM

Jennie-O Turkey Store, Willmar, MN 56201

Success in NAE (no antibiotics ever) production systems within the poultry industry is correlated to disease control. In the broiler industry there is a strong correlation to the success of such a program with a downtime of at least 14 days. In the turkey industry, there is a lack of data to support this correlation between downtime and NAE program success. In the current study, NAE test flocks with an increased downtime in finish were compared to NAE control flocks of a normal downtime for the production system. Measurements included environmental samples to measure microbiome changes, morbidity/mortality, serology for selected diseases, pre-move weights, and market performance parameters.

Pullet Vaccination Evaluations: the Good, the Bad, and the Ugly

Timothy Cummings

Zoetis

Poultry companies have designed intricate vaccination programs for their broiler breeder programs for two very important reasons; 1)to protect the life and health of the broiler breeder itself to maximize egg numbers, and 2) to pass along critical humoral antibodies to the broiler offspring against several significant pathogens which await them in the field. As such, the administration of the various live and killed vaccines to the pullets must be done properly to optimize the immune response and to get most value and protection from the vaccines themselves. Traditionally, the industry has utilized serology, audits, and emphasized training to the vaccinator crews to get this job done. The author has visited various poultry integrator complexes as a service to assess pullet vaccination crews and their techniques. This has afforded the opportunity to observe various processes and techniques in the field while learning a few things along the way. Presentation will focus on unique ways to help assess pullet vaccination procedures as well as offer the presentor's insights or observations on his findings.

Comparison of The Circadian Rythm Of Broiler Brreders And Commercial Layers During Lay On Different Biochemical Parameters With An Emphasis On Shell Formation

Daniel Venne[a], Marie Souvestre[b], Sezny Gall[b], Chloé Guilloton[b], Bastien Pradel[b], Jean-Pierre Vaillancourt[c]

[a]Couvoir Scott ltée, 1798 Kennedy, Scott (Québec), G0S3G0
[b]École Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles – BP 87614 – 31 076 – Toulouse Cedex 3 – France
[c]Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe (Québec), J2S 7C6, Canada.

Over the past several decades, modern chickens have been selected for two mutually exclusive specialized productions: meat production or egg production. This selection pressure has resulted in different adaptations according to feed management and egg production requirements. This paper will compare different biochemical parameters including blood pH, pCO2, HCO3, ionized calcium, total calcium, phosphorous, glucose, total protein, albumin and globulins between commercial layers and broiler breeders during a 24 hour cycle. Results show significant differences between breeders and layers and significant differences according to time post oviposition. Oviposition thus can be an easy time marker in the interpretation of results. Also as an example, calcium changes in commercial layers are more dependent on medullary bone mobilization as seen with significant changes in blood phosphorous levels and is more dependent on albumin transport that changes significantly in broiler breeders. Both type of birds regulate their blood bicarbonates to be at their peak during egg shell formation without significant changes in blood pH or PCO2. Further knowledge of these physiological changes may help in evaluating new nutritional and management strategies to help egg shell quality and reduce bone mining in older birds.

Posters
Antimicrobial

Reducing Microbial Load on Hatching Eggs Using a Dry Hydrogen Peroxide Gas System

[1]Julia McElreath, [2]Miguel Barrios, [1]Brian Jordan

[1]University of Georgia, Synexis [2]Biodefense Systems

An alternative method for egg sanitation could be of great value for the poultry industry. Preliminary data has indicated that a dry hydrogen peroxide (DHP) system can prevent growth or reduce microbial load on hatching eggs after exposure. Previous studies show reduced total microbial load on hatching eggs after 72 hours of exposure in a controlled laboratory setting. The objective of this study was to determine if prolonged exposure to DHP can reduce microbial load on hatching eggs in an animal research facility setting more similar to a commercial facility.

Efficacy of sodium formate (Amasil NA, monobutyrin, other glycerides, and glycerol (SiloHealth 104),) and bacitracin methylene disalicylate (BMD) on intestinal and processed parts bacteria count when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp).

Mike Coelho[1], Peter Ader[2]

[1]BASF Corporation, Florham Park, NJ 07932
[2]BASF SE, Lampertheim, Germany

A total of 3,744 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 9 replications). Birds were blocked by weight and sex. The treatments were T1=PC, no additive, no Cp; T2=NC, no additive, Cp; T3=NC +4/4/4 kg/MT SiloHealth 104 (SH) in starter/grower/finisher; T4=NC+ 4.0/2.5/1.5 kg/ MT SH; T5=NC+2.5/1.50/0.75 kg/MT SH; T6=NC+8/8/8 kg/MT Amasil NA (NA); T7=NC+2.5 kg SH + 4.0

kg/MT AN/ 2.5 kg SH + 4.0 kg/MT AN/2.5 kg SH + 4.0 kg/MT AN; and T8=NC+ Coccidiostat+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1-8 had litter inoculated on D0 with 2,500 oocytes of E. acervulina and E. maxima/bird, and T2-8 had litter inoculated with 5*104 CFU/bird of E. coli and 5*104 CFU/bird of Cp. On D21 and 42, 3 birds from each pen (27 birds/treatment) were sacrificed and data collected on Small Intestine (SI) and caecal (C) bacteria. At 43-45 days of age, 10 birds (5M and 5F) were processed from each replicate (9-replicates), to determine lesion scores and bacteria count. D21 SI lesion scores (0-3) (0.204, 1.667, 1.019, 1.037, 1.333, 0.778, 0.667 and 0.796, P<0.05, respectively): D21 SI Clostridium (CL) (3.595, 4.519, 4.209, 4.136, 4.388, 3.954 and 3.683 CFU log 10, P=<0.05, respectively); D21 SI Campylobacter (CA) (3.343, 4.489, 4.243, 4.074, 4.414, 3.834, 3.576 and 3.956, P<0.05, respectively); D45 processing breast salmonella incidence (1.111, 25.556, 13.333, 18.889, 15.556, 10.000, 7.778 and 6.667 %, P<0.05, respectively). In conclusion, monobutyrin, sodium formate and BMD significantly reduced bacteria count in small intestine, caecal and processing breast versus NC.

Are You Ready for an FDA Inspection? Ensuring a Valid VCPR for Poultry Prescriptions and Protocols

Steven Clark[1]; Tyler Holck[2]

[1]Poultry Veterinarian, Devenish Nutrition, LLC, 599 Chestnut Creek Rd, West Jefferson, NC 28694 (USA); [2]Veterinarian, GlobalVetLINK, 2604 Northridge Parkway, Ames, IA 50010 (USA)

Implementation of FDA Guidance Document #2131 on January 1, 2017 emphasizes more veterinary oversight of medically important antibiotics used in food producing animals. Only veterinarians with a valid veterinarian-client-patient-relationship (VCPR) can prescribe antibiotics deemed by FDA as "medically important". With this emphasis, there is a need to clarify the roles and responsibilities of veterinarians in establishing a valid VCPR, providing clients with compliant protocols, prescriptions, and veterinary feed directives (VFDs). Veterinary instructions, treatment plans, or protocols, are an excellent tool to communicate the veterinary instructions for health intervention and serve as a compliance document for one element of the VCPR. Another requirement of the VCPR describes the general or preliminary diagnosis of the medical condition requiring treatment. Electronic software solutions, such as, from GlobalVetLINK, LLC (GVL®, Ames, IA), reduce time spent on VFDs and scripts, reduce or eliminate paperwork, help enable accuracy of drug label information, verify completeness of certificates and assist with regulatory compliance. The included Table is a comprehensive list of FDA approved medications for poultry, indicating chicken/turkey approvals, current availability status, VFD or Non-VFD for feed medications, Prescription or Non-Script for drinking water drugs.

Prevalence and antimicrobial resistance of fecal *E. coli* and *Salmonella* isolates obtained from Ontario small poultry flocks

Csaba Varga[a], Marina Brash[b], Durda Slavic[b], Patrick Boerlin[c], Nancy Brochu[c], Michele Guerin[d], Leonardo Susta[c]

[a]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada, N1G 2W1
[b]Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[c]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[d]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Although keeping small poultry flocks is increasingly popular in Ontario, information on the prevalence of zoonotic foodborne pathogens and their antimicrobial susceptibility is lacking. A study of small poultry cases submitted by veterinarians to the Animal Health Laboratory, University of Guelph, was conducted between October 2015 and September 2017. From each submission a pooled cecal sample was obtained and tested for the presence of fecal *E. coli* and *Salmonella*. From each positive sample 3 isolates were selected and tested for antimicrobial susceptibility to 14 antimicrobials using a broth microdilution technique and the

Sensititre NARMS Gram-negative plate. Cases from 160 small flocks, consisting of chickens (84%), turkeys (6%), ducks (6%), and game birds (5%) were received. A total of 433 fecal *E. coli* and 15 *Salmonella* isolates were recovered. *Salmonella* serotypes isolated were *S*. Anatum, *S*. Indiana, and *S*. Ouakam (3 chicken submissions, 3 isolate each), *S*. Uganda (1 turkey, 3 isolate), and *S*. Montevideo (1 duck, 3 isolate). Two hundred and one *E. coli* isolates were pan-susceptible. Tetracycline (45 %), streptomycin (29 %), sulfonamides (18%), and ampicillin (16%) were the most common resistance found in *E. coli*. *Salmonella* isolates were most commonly resistant to streptomycin, tetracycline, sulfonamides and trimethoprim–sulphamethoxazole. No or low resistance among fecal *E. coli* and *Salmonella* isolates were detected to cephalosporins, carbapenems, macrolides and quinolones, antimicrobials important for human medicine.

Our results provide baseline data which can be used by future studies to measure differences in resistance following the upcoming changes in antimicrobial use legislation.

Antimicrobial resistance patterns in *Campylobacter* species isolated from poultry production facilities in Alberta

Karen Liljebjelke[1], Tam Tran[1], Niamh Caffrey[1], Yu Yu Wang[1], Rashed Cassis[3], Chunu Mainali[3], Sheryl Gow[4,5], Agnes Aguno[6], Sylvia Checkley[1,2]

[1]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Dr. NW, Calgary, Alberta, T2N 4Z6

[2]Provincial Laboratory for Public Health, Alberta Health Services, 3030 Hospital Dr. NW, Calgary, Alberta, T2N 4W4

[3]Alberta Agriculture and Forestry, 116 Street, Edmonton, Alberta, T6H 4P2

[4]Public Health Agency of Canada, 52 Campus Dr., Saskatoon, SK S7N 5B4

[5]Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Dr, Saskatoon, SK S7N 5B4

[6]Public Health Agency of Canada, Center for Foodborne, Environmental and Zoonotic Infectious Diseases, 160 Research Lane, Unit 103, Guelph, ON N1G 5B2

Campylobacter species *C. jejuni* and *C. coli*, are common causes of food borne diarrheal illness in people in the United States and Canada. Although most infections are mild and self-limiting, antibiotic treatment may be necessary, and antimicrobial resistance may result in poor outcomes. The aim of this study was to examine the antimicrobial resistance patterns of sixty-five Campylobacter isolates from seventeen poultry flocks in Alberta during 2015 – 2016. The isolates were collected as part of the Canadian Integrated Program for Antimicrobial Resistance (CIPARS). Chicks originated from three hatcheries, although 80% were from a single hatchery. Resistance to one or more antimicrobials assayed was detected in 9 of 17 flocks. Fifteen flocks reported use of bacitracin (n = 14), salinomycin (n = 9), or monensin (n = 5). Two flocks reported no antimicrobial use. Among 2015 isolates 29% (12/41) were multi-drug resistant (≥ 3 drug classes). No 2016 isolates were multi-drug resistant. Thirty percent of 2015 isolates had the resistance pattern:

Azithromycin/clindamycin/Erythromycin/Telithromycin. The *cme*B gene which encodes a multi-drug transporter, was identified in these isolates using PCR. This multi-drug resistance phenotype was transferred to recipients by conjugation in vitro. The presence of conjugative plasmids conferring multi-drug resistance among *Campylobacter* in poultry production is a public health concern.

Avian Influenza

Risk assessment of avian influenza virus dissemination in duck farms in France

Kateri Bertran, Luc Robertet, Alexandre Vove, Hugues Duret, Alize Mouchez, Jean-Luc Guerin

ENVT, INRA, UMR IHAP, Chair for Avian Biosecurity, University of Toulouse, 23 Chemin des Capelles, 31300 Toulouse

Since 2015, highly pathogenic avian influenza (HPAI) outbreaks have been reported in wild and domestic birds in Europe, France being one of the most affected countries. Duck farms have been particularly susceptible to HPAI virus, with dramatic economic consequences. Surveillance and control rely on termination of HPAI virus-infected flocks, and intensive surveillance of low pathogenic AI (LPAI) viruses before transportation to the slaughterhouse, with stricter biosecurity measures to reach slaughter. Here we evaluated the infection dynamics of LPAI viruses in ducks at the individual and flock levels to estimate the risk of viral spread. Mule duck farms from high-density duck-populated regions of France were considered. Flocks ready to enter the force-feeding stage and positive for M-gene PCR were selected. The target sample was 10 flocks (n=20 ducks/flock, N=400 ducks), each flock divided into 2 feeding units. Ducks were housed in collective cages and labeled. The following samples were collected at the beginning (day 0), middle (day 5), and end (day 11) of force-feeding: tracheal (TR) and cloacal (CL) swabs and feathers to evaluate individual virus shedding, serum samples to assess individual serologic status, and environmental (surfaces) swabs and manure samples from cages in the feeding rooms to determine environmental contamination and persistence. The majority of the selected flocks (8/10) had a short viral shedding persistence, with 0-5% positive TR swabs and 0-15% positive CL swabs at the beginning of force-feeding (day 0). However, 2/10 flocks had 35-50% positive CL swabs on day 0, some of which persisted in subsequent time points. Serology indicated that most of the ducks had had previous exposure to AI virus. These results contribute to better understand the infection dynamics of currently circulating LPAI viruses in duck farms in France, and provide the necessary data to improve AI surveillance and management.

Prevention and Control of Avian Influenza Through Biosecurity, Surveillance, Early Detection, and Rapid Response: It Is Easier Said Than Done

Nathaniel Tablante

University of Maryland College Park

The prevention of avian influenza (AI) is best achieved through biosecurity, surveillance, and early detection, while control is primarily accomplished though rapid response in case an outbreak occurs. Biosecurity is a well-known and long–practiced standard operating procedure (SOP) in the poultry industry but prone to vulnerabilities as evidenced by a number of major HPAI outbreaks in several poultry-producing countries including the U.S. A strong complement to biosecurity is surveillance and early detection through flock health monitoring, regular collection of samples such as tracheal swabs, and thorough testing for AI virus using rapid antigen capture tests or real-time RT-PCR. If an outbreak of AI occurs, rapid response within 24 hours through depopulation and carcass disposal is critical in stopping the spread of AI virus. Although poultry companies and animal health agencies have written protocols and procedures in place to prevent outbreaks of AI and other economically important poultry diseases, those who have experienced an actual outbreak of AI know that what looks good on paper does not always work in an actual field situation. There is no such thing as a “one size fits all” program. In order to effectively prevent and control AI, it is important to learn from previous outbreaks and adjust prevention and

control programs based on local conditions and available resources.

Outbreaks of Low Pathogenic Avian Influenza H7N3 in Turkeys in California.

H. L. Shivaprasad[a], J. Blue[b], G. Senties-Cue[b], S. Stoute[b] and Beate Crossley[c]

California Animal Health and Food Safety Laboratory System –[a]Tulare, [b]Turlock and [c]Davis branches, University of California, Davis

Avian Influenza (AI) is a viral disease of various species of birds that can cause respiratory signs, drop in egg production and variable mortality in turkeys, chickens, quail, *etc*., depending on the pathogenicity of the virus, low (LPAI) or highly pathogenic (HPAI) virus. An outbreak of AI occurred during September 2018 in 15-week-old turkeys in a flock of 7000 in central valley of California. Clinical signs included respiratory signs with swollen sinuses and increased mortality. Pathology of six turkeys revealed conjunctivitis, sinusitis, tracheitis and pneumonia. PCR of oropharyngeal swab and immunohistochemistry were positive for AI. AI virus was sequenced and found to be LPAI H7N3. Several weeks later another similar outbreak occurred in 10-week-old turkeys and the disease was detected in three other ranches by surveillance. In 2015 there was also an outbreak of LPAI H7N3 in turkeys. Information on surveillance, epidemiology, virology and disposal of birds will be presented and discussed.

Vaccine Protection of Commercial Broilers Vaccinated with an Avian Influenza Vector Vaccine *r*HVT-H5 and an Inactivated H5N2 Vaccine and Challenged with the Mexican H5N2 Highly Pathogenic Avian Influenza Virus (HPAIV)

Luiz Sesti[1], Andrés Soto[2], Rocio Fernandez[2], Samuel López[2], Vilmos Palya[3]

Ceva Animal Health, [1]Latin America, [2]Mexico, [3]Hungary

Commercial broilers positive to maternal antibodies against avian influenza H5N2 viruses were simultaneously vaccinated (SC) at day one with a vector *r*HVT-H5 vaccine expressing the hemagglutinin H5 antigen and an inactivated H5N2 vaccine (official 2015 strain). Vector vaccine-take was monitored in wing feather pulp samples taken at 21 post-vaccination (10 birds per group). The experimental birds (two vaccinated/challenged groups [15 birds each] and two non-vaccinated control/challenged groups [15 birds each]) were challenged (via unilateral nasal instillation) at 28 days of age with a HPAIV H5N2 isolated in Mexico in the 90's. Clinical behavior of challenged birds (mortality) was evaluated for 14 days post-challenge. Blood samples for H5 serology (HI) were taken (from 10 birds in the vaccinated groups and 5 birds in the control groups) at day 1 of age, 28 days of age, at challenge (35 days) and at 14 days after challenge (49 days) from all surviving birds. Challenge virus excretion was measured by *q*RT-PCR from individual oro-nasal swabs (choanal slit) taken at 2, 5 and 10-days post-challenge from 10 birds per group in all groups. Data on the clinical performance, challenge virus excretion and serology will be presented and discussed.

Chimeric virus-like particle vaccine elicits strong antibody response against different clades of H5 highly pathogenic avian influenza viruses

Jae-Keun Park[a], Jin-Yong Noh[a], Seong-Su Yuk[a], Jung-Hoon Kwon[a], Chang-Seon Song[a], Dong-Hun Lee[b]

[a]Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea.
[b]Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA

Asian-lineage H5 highly pathogenic avian influenza (HPAI) viruses have evolved independently into diverse, genetically and antigenically distinct hemagglutinin (HA) clades. We developed chimeric mono-clade and multi-clade HA virus-like particle (VLP) vaccines using the insect cell expression system, including clade 1 VLP, clade 2.3.2.1 VLP and clade 1/2.3.2.1 VLP. Specific pathogen-free chickens and commercial ducks were immunized with oil emulsion mono-clade and multi-clade VLP vaccine,

and the antibody responses were analyzed using the cross-clade hemagglutination inhibition test. Chickens immunized with mono-clade VLP vaccine showed significantly higher cross-clade immune responses than ducks. Ducks immunized with mono-clade VLP vaccine showed little or no cross-clade immune responses. Immunization with chimeric VLP vaccine induced production of a significantly broader spectrum of antibodies against different clades of viruses in both chickens and ducks. These results suggest that utilization of chimeric VLP technology in poultry species would be a promising strategy for the control of Asian-lineage HPAI viruses.

Genetic Characterization and Transmissibility of the First H9N2 Avian Influenza Virus in Indigenous Chickens from Kenyan Live Bird Markets

Henry M. Kariithi[† 1, 2], Catharine N. Welch[† 2], Elizabeth A. Pusch [2], Leonard O. Ateya [1], Yatinder S. Binepal [1], Auleria A. Apopo [3]; Jacqueline K. Lichoti [3], Helena L. Ferreira [2, 4], David L. Suarez [2] and Claudio L. Afonso [2]

[1] Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, P.O Box 57811, 00200, Kaptagat Road, Loresho, Nairobi, Kenya;
[2] Southeast Poultry Research Laboratory, US National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, 934 College Station Road, Athens, GA, 30605, USA;
[3] Directorate of Veterinary Services, State Department of Livestock, Ministry of Agriculture, Livestock and Fisheries, Private Bag-00625, Nairobi, Kenya;
[4] University of Sao Paulo, ZMV- FZEA, Pirassununga, 13635900, Brazil
† Authors with equal contribution

Avian influenza viruses (AIVs) have been reported in various avian species in Kenya since 2009. Here, we report the first characterization of an H9N2 low pathogenic avian influenza virus (LPAIV) in the country. Oropharyngeal swabs (n=282) collected in 2017 and 2018 from backyard and live bird markets (LBMs) indigenous chickens were screened by AIV-specific real time RT-PCR (RRT-PCR), followed by virus isolation, MiSeq sequencing and phylogenetic analysis. Infection susceptibility was experimentally determined by nasal-inoculations of three-weeks-old SPF chickens with low and medium doses of a representative of the AIV isolates. Virus transmission was determined by exposure of naïve, uninfected birds to the inoculated chickens at two days post inoculation (dpi). Virus shedding was quantified from swabs (2, 4, and 7 dpi), and seroconversion evaluated at 14 dpi. The RRT-PCR detected AIV infection in 12 LBM samples, five of which were isolated and sequenced. Phylogenetic analysis confirmed that the isolates belonged to the G1 lineage of H9N2 LPAIV, which showed nucleotide sequence identity (98.6-99.9%) to a 2017 Ugandan H9N2 isolate. Susceptibility and transmission experiments resulted in neither mortalities nor clinical signs in any of the H9N2-inoculated and contact birds, and all birds shed high virus titers, except the contact birds at 2 days post exposure. Birds from all groups seroconverted at 14 dpi/12 dpp, confirming H9N2 LPAIV infection and transmission. Since LBMs serve as sources for restocking to rural free-range poultry, future studies should determine AIV genotypes, their prevalence and endemicity in Kenya.

Pathogenicity and Transmission of Clade 2.3.4.4 H5Nx Highly Pathogenic Avian Influenza Viruses in Chickens.

Jung-hoon Kwon[a], Dong-hun Lee[a, b], Miria Ferreira Criado[a], Kateri Bertran[a, c], David E. Swayne[a]

[a]Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA, USA;
[b]Current address: Department of Pathobiology & Veterinary Science, University of Connecticut, Storrs, CT, USA;
[c]Current address: Université de Toulouse, ENVT, INRA, UMR 1225, 31076 Toulouse, France

H5Nx clade 2.3.4.4 highly pathogenic avian influenza viruses (HPAIVs) have evolved into four distinct subgroups (A–D) and spread to various geographic regions by wild birds. Further, these

viruses evolved into various novel genotypes by reassortment with local low pathogenic avian influenza viruses. To determine if the biological characteristics have changed for these genetically divergent clade 2.3.4.4 viruses, the pathogenicity and transmissibility of the clade 2.3.4.4 subgroup A-D were evaluated in SPF chickens using the seven strains representing each of the four subgroup. The birds inoculated with subgroup B and C viruses showed 100% mortality. However, slightly lower mortality was detected in the birds inoculated with subgroup A (80%) and D (90%) viruses with delayed mean death times. The subgroup C viruses efficiently transmitted to all three co-housed birds, but only two of three birds co-housed with subgroup A and D inoculated birds died, respectively. All birds co-housed with subgroup B inoculated birds lacked clinical signs and survived. These results indicated that the subgroup A, B and D viruses used in this study were not optimally adapted to chicken as compared with subgroup C viruses. Since novel genotypes of clade 2.3.4.4 HPAIVs continue to emerge, pathogenicity and transmission studies are an ongoing necessary to understand and mitigate the changing risk of HPAIVs to the global poultry industries.

Development and comparison of two primary chicken tracheal cell culture systems for the study of avian influenza virus infection

Emily J. Aston[a], Rodrigo A. Gallardo[b], Huaijun Zhou[a]

[a]*Department of Animal Science, College of Agricultural and Environmental Sciences, University of California-Davis,*
[b]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis*

Avian influenza virus (AIV) initially establishes infection in the cells of the respiratory epithelium. Here we develop two culture systems consisting of primary tracheal epithelial cells derived from chicken embryos and compare their use in AIV infection studies. A traditional cell culture system is comprised of a cell monolayer submerged in media and has been used successfully for *in vitro* infection studies with avian respiratory viruses. In an air-liquid interface (ALI) culture system, the apical surface of the cell layer is exposed to air while the basal surface is submerged in media. The ALI system supports mucociliary differentiation, which resembles the airway both morphologically and physiologically, and is increasingly used in both human and veterinary research but has not yet been described for chickens. Primary tracheal epithelial cells were harvested from 18-day-old chicken embryos and seeded on tissue culture plates for traditional culture and membrane supports on Matrigel®-coated plates for ALI culture. *In vitro* infections with AIV were performed on traditional cultures following attachment (at least 24 hours) and fully-differentiated four week-old ALI cultures. Infection of the epithelial cell cultures with AIV was demonstrated using immunofluorescence, and AIV growth curves were performed to confirm that the cells could support AIV growth and reinfection. The establishment of a primary tracheal cell culture system that closely mimics the environment in the chicken respiratory tract may increase the biological relevance of *in vitro* avian respiratory virus infection studies to enhance our understanding of molecular mechanisms of disease resistance to AIV in chickens.

Extended laboratory exercise on surge capacity of highly pathogenic avian influenza (HPAI) virus testing

Stephanie Forrester, Arun Kulkarni, and Len Chappell

Georgia Poultry Laboratory Network, 3235 Abit Massey Way, Gainesville, GA 30507

Laboratory diagnosis of avian influenza has become a cornerstone for the prevention, containment, and surveillance strategies of this disease. During a suspected or actual outbreak of highly pathogenic avian influenza (HPAI), the key goals of response are first to meet the surge requirements for diagnostic testing and secondly, to report all diagnostic test results to appropriate personnel and information management systems as soon as possible. Georgia is one of the leading poultry-producing states in the U.S. An occurrence of HPAI could be devastating to the local and state economy. It has been estimated

that in a typical hot zone of two miles there could be over 410 houses with a total bird population of 8.44 million. Previously we showed that during a ten hour shift we were able to process four hundred randomly spiked specimens. This testing capacity will be inadequate to accommodate the poultry population of this size in addition to anticipated increase due to continuity of operations. In this exercise, we reevaluated the current surge-capacity model, to determine if additional sample flow can be handled during two shifts. The results of seven hundred randomly spiked inactivated AIV specimens will be presented using two different USDA-approved platforms.

Bacteriology

Characterization of the duodenal microbiota in egg layers with Focal Duodenal Necrosis using next generation sequencing

Monique França[a], Ana M. Villegas[a,b], Lisa Stabler[a]

[a]Poultry Diagnostic and Research Center, Department of Population Health, University of Georgia, Athens, GA 30602.
[b]Department of Poultry Science, University of Georgia, Athens, GA 30602

Focal duodenal necrosis (FDN) is an intestinal disease that affects table egg layers. Previous studies have associated this condition with different *Clostridium* species; *C. colinum* and *C. perfringens*. The objective of this study was to characterize the duodenal microbiota of egg layers affected with FDN. Duodenal samples from healthy and FDN-affected egg layers were collected for microbiota analysis. Genomic DNA was extracted from duodenal samples and next generation sequencing was performed to target the bacterial 16S rRNA. Significant differences were detected in the microbiota composition between samples with FDN and samples obtained from healthy birds. Two studies were conducted and revealed similar findings. *Lactobacillus*, *Helicobacter* and *Tyzzerella* were the predominant Genera found in FDN-affected duodenal samples in both studies. The genus *Tyzzerella* was significantly more abundant in FDN-affected duodenal samples. *C. colinum* and *C. piliforme* are closely related bacteria in the *Tyzzerella* genus. PCR for *Clostridium colinum* and *Clostridium piliforme* was performed in *Tyzzerella*-positive FDN samples and yielded negative results. Additional bacteriological studies are needed to try to further characterize, identify and isolate the *Tyzzerella* bacteria found in association with FDN.

Molecular Characterization of Avian Pathogenic *Escherichia coli* (APEC) from Turkey Cellulitis.

Catherine M. Logue[a], Aline De Oliveira[a], Lisa K. Nolan[a] and Nicolle L. Barbieri[a]

[a]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens GA.

Colibacillosis is a significant cause of economic loss to the world's poultry industry as a result of morbidity, mortality and carcass condemnation. Coliform cellulitis caused by Avian Pathogenic *Escherichia coli* (APEC) is characterized by local inflammatory exudates in the subcutaneous tissues. In turkeys, it often afflicts near market age Toms. Here, we characterized APEC isolated from cellulitis lesions of turkeys, systemic isolates recovered from internal organs of infected turkeys and isolates recovered from used litter. Isolates were compared by assignment to phylogenetic groups, possession of virulence traits (chromosomal and plasmid) and antimicrobial resistance. There were also profiled using PFGE and molecular serogrouping. Phylogenetic groups A and B2 were some of the most common phylogenetic groups detected with greatest levels of B2 (a pathogenic type) found in systemic isolates (30.7%). Screening for virulence-associated genes including those of the ColV plasmid which are considered a defining trait of APEC, found that genes of the conserved region of ColV plasmids had a higher prevalence in systemic and cellulitis isolates (46-61%) compared to litter isolates. Similarly, genes encoding resistance to heavy metal compounds such as copper, arsenic and zinc (35-85%) were more prevalent in isolates from disease than litter. Serogroups of *E. coli* detected included O2; O8; O25 and O50. Ongoing work is assessing the molecular relationship between *E.*

coli-associated cellulitis in turkeys and *E. coli* of litter to determine the contribution of litter contamination to the disease.

Complete Genome Sequence of Mycoplasma iowae (MI) type strain 695

Mohamed El-Gazzar[a] and Mostafa Ghanem[b],

[a]Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.
[b]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA

Mycoplasma iowae (MI) is one of the four pathogenic mycoplasma species in poultry. It was first isolated in the state of Iowa, USA in 1955, to be later characterized by Yoder and Hofstad (1964). Clinical signs of MI infection in turkey include decreased hatchability, mostly due to late embryo mortality. It may also lead to leg deformities and joint infection in the first two weeks of age resulting in poor flock uniformity and delayed growth. Availability of a complete genome sequence is very important to further our knowledge of the genome structure, functions, and dynamics. Currently, there is no complete genome sequence available for MI. In this study, we are proposing that the use of an advanced long-read sequencing technology like PacBio Single Molecule, Real-Time (SMRT) could help in generating a complete genome sequence for MI. We have selected the MI type strain 695 to be sequenced using this technology on PacBio RSII. The generated raw reads was assembled using Hierarchical Genome Assembly Process (HGAP.3). The assembled genome consisted of one contig of 1,315,491 bases with an average coverage of 310.5X. The complete genome sequence was submitted to GenBank with the accession number CP033512.The new genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). This genome opens the door to improve our understanding of many biological insights of this pathogen including pathogenic mechanisms, virulence factors and immune dominant antigens. In addition, it helps in developing robust genotyping schemes for epidemiological investigations.

Investigation of outbreaks of infectious endocarditis in mule duck: epidemiological study and potential etiological role of *Streptococcus pluranimalium*

Jean-Luc Guerin[a], Marie Souvestre[a], Luc Robertet[a], Sandrine Castaing[b], Edouard Huchin[c], Laurent Deffreix[b], Léni Corrand[e], Damien Dubois[f], Guillaume Croville[a], Marie-Noëlle Lucas[a], Maxence Delverdier[a], Eric Oslwad[f]

[a]ENVT, INRA, ENVT 23, chemin des Capelles, 31076 Toulouse, France
[b]Biovol 47 - Reseau Cristal, Vignes de la justice, 47150 Monflanquin, France
[c]Abiopole, Cabinet veterinaire, 19270 Ussac, France
[d]SOCSA Elevage 40, 281 avenue du B´earn, 40330 Amou, France
[e]Anibio, ENVT, ENVT 23, chemin des Capelles, 31076 Toulouse, France
[f]Service de Bacteriologie, CHU Toulouse Purpan, 31100 Toulouse, France

Streptococcus pluranimalium infection, associated with septicemia and valvular endocarditis, has been described in broiler chickens. Fourteen batches of mule ducks from various departments of South-West, France, and without apparent epidemiological links, were included in a clinical study from January to March 2018. All showed an increased mortality on birds aged 15 to 58 days. At necropsy, splenomegaly and associated spleen necrosis, marked vegetative endocarditis, pericarditis were observed. At histopathology, the picture included septic vegetative endocarditis, hepatic and / or pulmonary congestion, and myocardial and splenic infarction (septic thromboembolism). The bacteriological examinations revealed *Streptococcus pluranimalium* in 14 batches of mule ducks. Extraction was performed from vegetative endocarditis lesions on the mitral valves. A PCR targeting 16S gene was performed on ducks from two batches with lesions and high mortality. Subsequently, all strains isolated in the field were typed by Maldi-Tof mass spectrometry and sequencing of the *SodA* gene. Molecular analyzes were performed in parallel to detect any immunodepressive virus (Derzsy's parvovirus, duck

circovirus, goose polyomavirus): 6/9 batches were detected positive for the Derzsy disease virus. Sequencing of the 16S gene confirmed, in the two cases studied, that *S. pluranimalium* was detected in abundant quantities on the mitral valves. Sequencing and assembly of the complete genome of 2 isolates was performed using NGS and confirmed the identification. In the future, inclusion of new field cases and experimental infection would help to better understanding the pathogenesis of *S. pluranimalium* infection in birds.

Pathogenecity of Avibacterium paragallinarum Isolates with Different Nicotinamide Adenine Dinucleotide Requirements of South Korea

Ok-Mi Jeong

Animal and Plant Quarantine Agency

Infectious coryza(IC) is an acute respiratory disease of chickens caused by Av. paragallinarum. It causes economic losses from retarded growth and reduction in egg production in layers. Normally, Av. paragallinarum requires NAD for growth. Recently we isolated four different NAD requiring isolates. The present study was to determine the virulence of those isolates that require more or no NAD for growth.

Effect of a Feed Incorporated Phytobiotic on Intestinal Lesions in Poultry Challenged with *Clostridium perfringens*

Igor Praxedes-Campagnoni [1,3,4], Fabio Gazoni [3], Bruno Vecchi [2], Sherry Layton[1,2]

[1]BV Science, 8651 Highway N, Lake St Louis, MO USA
[2]Vetanco SA, Chile 33, Buenos Aires, Argentina
[3]Vetanco Brazil, Chapecó, Santa Catarina, Brazil
[4]University of Santa Catarina State, Department of Animal Health, Santa Catarina, Brazil

Phytobiotics can address pathogen challenges in the poultry industry and can be used as novel growth promoters. The aim of this trial was to evaluate a metallo-phytobiotc (MP) to control *Clostridium perfrigens* (CP) *in vitro* and *in vivo*. *In vitro* microdilution analysis was performed to determine minimal inhibitory concentration (MIC) of MP against CP. Once MIC was determined, 100 day of hatch male chicks (Cobb 500) were divided and randomly assigned to 1 of 5 treatment groups housed in isolation cages. All chicks were fed a traditional corn-soy starter feed was for the initial 7 days and then replaced with a wheat (62.7%)-soy (29.6%) diet with treatment modifications: T1 non-treated/non-challenged, T2 non-treated/challenged. T3 MP 1kg/ton/challenged, T4 MP 0.75kg/ton/challenged, T5 0.5kg/ton/challenged. On d13 and 14 challenged groups received a commercial coccidia vaccine and a commercial Gumboro vaccine at 10X dose; d15 and 10 challenged groups were challenged with 108 CFU/ml of a netB+ Type A CP. At d 21 all birds were humanely sacrificed, intestines removed and intestinal lesions determined using Craven's lesion scoring 0-5 (where 0 is no lesions and 5 is severe lesions). Lesions scores for each group were as follows: T1:1.45b; T2: 2.25a; T3:1.65b; T4:1.80b and T5:1.95a (different letters represent significant difference; $p<0.05$). Data show there was lesions in all treatment groups; however, T3 and T4 (MP 1kg/ton and 0.75 kg/ton) had the same lesion score as T1 without challenge. This study provides promising evidence that in a dose dependent CP is inhibited by MP.

Mycobacterial Infection in a Pet Zebra Finch

Kristen Hill-Thimmesch[1], Tsang Long Lin[1], Tamantha Hickok[2], and Pat S. Wakenell[1]

[1]Purdue University Department of Comparative Pathobiology, West Lafayette, IN
[2]Animal Medical Center, Yorktown, IN

A 6-year-old, male, Zebra Finch was humanely euthanized following a two-week history of weakness and respiratory distress. Gross examination revealed few, scattered, white to tan nodules adhered to the visceral aspect of the sternum and right rib cage. Similar nodules were present on the left liver lobe and pericardial sac adjacent to the base of the heart. Histopathologic examination of the heart, lung, air sac, esophagus, crop, liver, spleen, skeletal muscle, testicle and

kidney found multifocal to coalescing areas of pallor composed of variably sized aggregates of plump macrophages with peripheralized nuclei and abundant, pale, granular cytoplasm. Histochemical stains (Periodic acid-Schiff, Gram and Ziehl-Neelsen) were performed in selected tissue sections. Periodic acid-Schiff stain was negative for fungi. Gram stain revealed weakly Gram positive bacilli in macrophages. Ziehl-Neelsen stain was positive for numerous acid-fast bacilli within macrophages. Based on the granulomatous inflammation combined with the presence of acid-fast bacteria, a diagnosis of avian mycobacterial infection was made. A sample of affected lung submitted to Athens Veterinary Diagnostic Laboratory for PCR and sequencing found *Mycobacterium genavense*. This organism is a slow growing, fastidious, non-tubercle forming mycobacterium that is considered the most common cause of avian mycobacteriosis in passerines and psittacines.

IDENTIFICATION OF GALLIBACTERIO ANÁTIS IN EGG PRODUCTION EGGS FOR PLATE USING THE PCR TEST IN THE ALTOS AREA OF JALISCO, MÉXICO

Alfredo Mercado[1] Alejandro Hori[2]

[1]Flemate Poultry Revival
[2]Galván Boehringer Ingelheim Animal Healt

The present work was carried out in a poultry unit. The state of the recurrent form has been presenting clinical problems attributable to an infectious process by Avibacterium paragallinarum, leaving aside the identification of other agents involved. A clinical behavior was followed, a flock of laying birds, which were immunized at 6 and 14 weeks of age, the first with a bacterium that prevents Coriza disease, which includes the three serovars A, B and C The second one also includes the serovars for antigenic music information to provide protection against Gallibacterium anatis. In both cases, the same vaccination and dose schedule was implemented to protect the rest of the diseases in the area. Likewise, evidence was sought in the presence of Gallibacterium anatis in the poultry farm through the real-time PCR test. The results using the PCR test indicate POSITIVE to the presence of Gallibacterium anatis after 30 weeks of age for the two flocks. This work has coincided in the moment in which the bacterium is presented and the presence of clinical signs that are published, published, published, published, and reported on the theme of Campogarrido and the collaborators in the publication.

Case Reports

Chicks exhibiting drunken behavior

Lara Lamoureux, Jean-Pierre Vaillancourt, Daniel Venne

University of Montreal

In October of 2018, a Quebec chicken grower expressed concerns about chicks that had just been delivered to his farm. Birds were staggering around and were not eating properly. Chicks weighed only 70 g on day 5, which represented only about 74% of expected weight at that age. Mortality was not an issue with only six dead out of 6000 by day 4. A post-mortem of these birds showed some minor hepatic lesions and sub-cutaneous oedema of the brain area. Aggregates of macrophages and eosinophils were observed in the liver. The most significant lesions were in the brain with the degenerescence of Purkinje cells. An investigation of the feed demonstrated that these chicks were fed 2.28 Kg/tonne of the anticoccidial zoalene, instead of the recommended 0.5 Kg/tonne (0.0125%). If presented in a talk, this case would also show biochemical blood results as well as videos of the observed clinical signs.

Mycotoxins Toxicity in Laying Hens

Pablo C. Morales-Cardenas[1], Omar Moreno-Hoyos[1], Alba R. Vasquez[1], Viviana Parada[2] and Andrés Rodriguez-Avila[3]

[1]Avicola El Guamito-Acebedo Silva S.A., [2]Facultad de Microbiología Industrial Universidad de Santander, Bucaramanga, SAN and [3]BioARA SAS Guaduas, CUN, Colombia

In March 2017, zootechnical parameters were observed below the expected average in the growing farm (flocks 549 and 550), in addition, an increase in mortality and selection in the following flocks was presented (551-53). The highest mortality peak was reached by flock 553 at 16 weeks of age with 9.5%. The affected chicks showed low feed intake, decreased water consumption, different degrees of paralysis, from gait alterations to inability to move. Different samples were taken for isolation, polymerase chain reaction (PCR) and histopathology. In addition, samples of feed and water to determine possible toxins. No virus was isolated, some secondary bacteria were isolated, only vaccine strains were detected by PCR, water quality was acceptable, the most commonly found lesion was hepatomegaly and high levels of mycotoxins were found in feed samples by high performance liquid chromatography (HPLC). Due to the low production parameters and the detection of mycotoxins, a mycotoxin binder was included in the feed in 41,000 chicks of flock 554, leaving 56,000 chicks as control of the same flock without any product in the feed. After 18 weeks of growth period, the mortality and selection of the pullets with the mycotoxin binder in the feed was lower than in the control group. Comparing egg production at week 23 of age the hens with mycotoxin binder had 4 eggs per bird housed more than those of the control group.

Chronic Mycosis in a Backyard Hen

Laura Morman, DVM, Avian Diagnostic Medicine Resident

Animal Disease Diagnostic Laboratory, Purdue University

A six month old Black Copper Maran mix hen was submitted to Purdue University's Animal Disease Diagnostic Laboratory for necropsy. The hen came from a backyard flock of five chickens. Clinical signs of lethargy, diarrhea, and anorexia had been treated for four weeks with courses of antiparasitic and antibiotic treatments. Necropsy revealed diffuse muscle atrophy, a focal pectoral granuloma, pulmonary nodules, and chronic, fibrous air sacculitis with fungal plaques. There was concurrent crop mycosis and caseous stomatitis. Lobules of the left kidney were grossly enlarged and both kidneys had a pronounced trabecular pattern with white, reflective foci within the parenchyma, suggestive of gout. Results of bacteriology and histology are pending; these will elucidate the causative agent and likely pathogenesis which lead to the diffuse, multifaceted infection.

Diagnostics

A summary of epidemic pattern of increased infectious bronchitis virus isolations in broiler and layer chickens in Pennsylvania

Carrington Stephenson[A], Lin Lin[A], Patrica Dunn[A], Eva Wallner-Pendleton[A], Brian Ladman[B], Huaguang Lu[A]

[A]Animal Diagnostic Laboratory, The Pennsylvania State University, University Park, PA; [B]Department of Animal & Food Sciences, University of Delaware Newark, Delaware

A growing number of case submissions requested for infectious bronchitis virus (IBV) isolations were seen at our laboratory during a four month period in late spring and summer recently. There was a total of 46 case specimen submissions that were suspicious of IBV infections of broiler and layer

chickens, which showed most clinical signs of increased air sacculitis in broiler cases and decreased egg production in layer cases. The submitted tissue specimens included tracheas, lungs, kidneys, and cecal tonsil, which were separately processed and each inoculated into 9-11 day old embryonating chicken eggs and IBVs were isolated after multiple egg passages were made. The chorioallantoic membrane of the final passage was tested by an IFA staining using anti-IBV group-specific monoclonal antibody. The virus isolation and IFA confirmation resulted in 31 IBV positive isolations of the 46 total cases. selected IBV isolates were processed for molecular characterization by sequencing S1 genes at the University of Delaware Poultry Health System (Newark, DE). The various S1 genotypes of the confirmed isolates included Mass, Conn, Delaware072/92, DMV/ 1639/11, and Georgia type viruses (GA08 and Folds). Our observation of this epidemic pattern in IBV diagnostics may alert a potential high prevalence of this disease thus we need to develop effective methods in response to its impact on poultry industry.

Identification and lineage typing of infectious bronchitis virus in clinical samples by real-time MinION Nanopore sequencing

Salman L Butt[1], Eric C. Erwood[1], Jian Zhan[1], Kelsey Young[1], Kevin K. Lahmers[2], Holly S. Sellers[3], James B. Stanton[1*]

[1.]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, 30602, USA
[2.]Department of Biomedical Sciences & Pathobiology, VA-MD College of Veterinary Medicine, Virginia Tech
[3.]Poultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, 30602, USA
**Corresponding author: James B Stanton*

Infectious bronchitis is a major cause of economic losses in global poultry industry. Part of the difficulty in controlling infectious bronchitis is that the causative agent (infectious bronchitis virus, IBV) has marked genetic diversity due to its high mutation rate and the ability to undergo genomic recombination. Additionally, inoculation with modified live vaccines can confound diagnostic tests. Collectively, these features create a need for classifying the varying IBV isolates and a diagnostic conundrum for interpreting PCR-based results in relation to a clinical problem in the field. Here we describe a MinION-based AmpSeq method that detected and genetically typed IBV from clinical samples. Total RNA was extracted from tracheal scrapings and oropharyngeal swab samples (n = 15), randomly reverse transcribed, and then PCR amplified using primers targeting the S1 segment of IBV. Amplicons were barcoded to allow for pooling of samples, processed per manufacturer's instructions into a 1D MinION sequencing library, and then sequenced on the MinION. Read files were processed through a pipeline including Albacore for basecalling, Porechop for demultiplexing and trimming, and Centrifuge and BLAST for read classification using custom databases. The AmpSeq method detected IBV in 13 out of 14 IBV positive samples. In 10 samples with multiple IBV lineages, this method accurately detected and genotyped IBV in 6 samples. These results were confirmed with genotype specific RT-qPCR and RT-PCR coupled with Sanger sequencing. The results demonstrate the feasibility of using MinION-based AmpSeq for rapid detection, accurate identification and lineage typing of IBV from clinical oral swab samples.

Poultry vaccines: innovative serological assays for diagnosis and vaccination monitoring for H5, H7 and H9 avian Influenza A

Lesceu Stephanie[1], Gaimard Marina[1], Mouacha Khadija[1], Redal Chloé[1], Druss Jean-Emmanuel[1], Lefebvre Catherine[1], Pourquier Philippe[1]

[1]IDvet, 310 rue Louis Pasteur, 34790 Grabels - FRANCE

Influenza viruses belong to the family *Orthomyxoviridae and* infect a variety of human and animal hosts. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further

divided into subtypes based on their Hemagglutinin (H) and Neuraminidase (N) antigens. Eighteen H antigens (H1 to H18) and eleven N antigens (N1 to N11) have been isolated. Most avian influenza viruses (H1 to 18 subtypes) are low pathogenic, such as H9, whereas some subtypes containing H5 and H7 are associated with highly pathogenic forms. Co-infection between avian respiratory diseases and low pathogenic H9 could lead to important losses in poultry flocks. For many years, inactivated vaccines based on circulating hemagglutinin or neuraminidase were developed to protect flocks against Influenza such as H5 and H7. As to control also H9 outbreaks, specific vaccines were developed leading to an increased need for rapid and reliable diagnostic and monitoring tools. Serological techniques are commonly used for disease monitoring. ELISA testing is an efficient and cost-effective method for the analysis of large numbers of samples, particularly in comparison with the Hemagglutination Inhibition Test (HI). As a result, IDvet has developed new tools to monitor vaccination uptake for H5, H7 and H9 AI: ID Screen® Influenza H5, H7 and H9 Indirect. IDvet's kits are quantitative tests and highly correlate with HI tests (homologous strain). They are the only commercial ELISAs able to detect H5, H7 or H9-specific antibodies for diagnosis and monitoring of vaccination (conventional and recombinant vaccines). *The following presentation summarizes the validation data obtained for Avian Influenza diagnostic tools developed by IDvet.*

Validation of Two New Real-Time Avian Influenza PCR Kits

Gwenllyan Slacum[a], Jantina De Vylder[b]

[a]BioChek USA Corp. 3 Southgate Road, Scarborough, ME 04074,
[b]BioChek B.V. Fokkerstraat 14, 2811ER Reeuwijk, Netherlands.

Over the last decades, the number of Avian Influenza outbreaks have increased drastically. The incidence and spread seem to have become so severe that endemic infections across the world have become a reality. One of the most effective ways to identify and monitor AI viruses in poultry is by use of a continuous global surveillance program. Recently, BioChek has developed 2 new real time PCR kits, an Influenza type A and an Influenza H5/H7/H9 multiplex qPCR kit, which can be used as diagnostic tools in large-scale AI surveillance programs. Both kits have been extensively validated against the Nagy and Spackman generic Influenza A detection assays and in house H5, H7 and H9 specific PCRs respectively by a UK reference laboratory. Testing for AI viruses was performed on a panel of 50 clinical samples from a low pathogenicity AI H7N7 outbreak and on a panel of 39 isolates covering all haemagglutinin and neuraminidase subtypes. In summary, the BioChek Influenza A PCR performs significantly ($P<0.000$) better than both the Nagy and the Spackman avian Influenza A virus M gene PCRs. The BioChek H7 PCR (as part of the multiplex kit) also performed better than the in house H7 PCR and equivalent performance was noted for H5 and H9 detection between the BioChek H5/H7/H9 multiplex kit and the in house H5 and H9 assays. Kits have become available outside the US to allow for rapid and cost-effective screening.

Optimization of The Protocol For Detection of Turkey Arthritis Reovirus by RT-PCR

Robert Porter, Zachary D. Bradley, Sunil Kumar, and Sagar Goyal

Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Turkey arthritis reovirus (TARVs) is a significant cause of lameness in turkeys, primarily affecting 12-17-week-old male turkeys; birds are recumbent with wing tip bruises, uni- or bilateral swelling of the hock and occasional gastrocnemius tendon rupture. In severe cases, lameness can affect 20-40% of the flock. We have reproduced the disease in turkeys, fulfilling Koch's postulates. In some instances, a reduced sensitivity of the RT-PCR to detect TARV in infected tendons may not be a primary assay issue, but rather an issue with sample collection. Currently, 3-6 legs are received at the MVDL (Minnesota Veterinary Diagnostic Laboratory). From these legs, 0.5 cm and 3.0 cm-length sections of gastrocnemius tendon and digital flexor tendon,

respectively, are pooled. The pooled samples are homogenized, centrifuged, and the supernatant used for virus isolation (egg and QT-35 cells) and RT-PCR. In our current study, single tendons from individual legs and pooled tendons were tested and compared. We hypothesized that testing the samples individually would enhance the sensitivity of the RT-PCR. Results indicated that there were cases where the RT-PCR on pooled tendons from multiple turkeys was negative, but leg tendons from individual turkeys were RT-PCR positive for TARV. Thus, there may be a dilution effect created by pooling tendons from multiple legs prior to testing.

Use of an Infectious Laryngotracheitis Virus gB Protein Elisa kit to Assess the Serological Response to Vaccination

Lozano Fernando[1], Leonardo Alvarado[1], Vilmos Palya[1], Stephanie Lesceu[2], Rafael Forero[2]

[1]Ceva Animal Health.
[2]Innovative Diagnostics ID.vet

The aim of this study was to evaluate the serological response to Infectious Laryngotracheitis (ILT) vaccination using a glycoprotein B (gB) of the ILT virus in an indirect ELISA kit. A total of 857 serum samples collected from birds at different ages post-vaccination from 12 commercial layer operations in Colombia were used for this evaluation. Commercial layer flocks vaccinated against ILT using different vaccination regimes comprising ILT-vectors and conventional live tissue culture vaccines were selected for this evaluation. Reference serum samples from SPF birds vaccinated with an ILT-vector vaccine were used for the comparison of the serological response of the commercial layer flocks used for this study. Serum samples collected included the sera from layer flocks which were affected by a field ILT virus. Results of this evaluation will be presented at the meeting.

MinION sequencing to genotype US strains of infectious laryngotracheitis virus.

Stephen J. Spatz[a]*, Maricarmen Garcia[b], Sylva Riblet[b], Teresa A. Ross[a], Jeremy D. Volkening[c] Tonya L. Taylor[a], Taejoong Kim[a] and Claudio L. Afonso[a]

[a]US National Poultry Research Center, Agricultural Research Service, United States Department of Agriculture, Athens, GA 30605, USA.
[b]Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, 953 College Station Rd., Athens, GA, USA
[c]BASE2BIO Oshkosh, WI USA

Over the last decade the US broiler industry has fought long-lasting outbreaks of infectious laryngotracheitis (ILTV). Previous nine genotypes (I-IX) of ILTVs have been recognized using the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method with three viral alleles (gB, gM and UL47/gG). In this study, the genotyping system was simplified to six genotypes by amplicon sequencing and examining discriminating single nucleotide polymorphisms (SNPs) within these open reading frames. Using phylogenomic analysis of 27 full genomes of ILTV, a single allele (ORFA/ORFB) was identified containing SNPs that could differentiate ILTVs into genotypes congruent with the phylogenetic partitioning. The allelic variations allowed for the cataloging of the 27 strains into 5 genotypes: vaccinal TCO, vaccinal CEO, virulent CEO-like, virulent US and virulent US backyard flocks from 1980 to 1990, correlating with the PCR-RFLP genotypes I/ II/ III- (TCO), IV - (CEO), V – (virulent CEO-like), VI – (virulent US) and VII/VIII/IX – (virulent US backyard flock isolates). With the unique capabilities of third generation sequencing, we investigated the application of Oxford Nanopore MinION technology for rapid sequencing of the amplicons generated in the single allele assay. This technology was an improvement over Sanger-based sequencing of the single allele amplicons due to a booster amplification step in the MinION sequencing protocol. Overall, there was a 90% correlation between the genotyping results of the single allele assay and the multi-allele assay. Surveillance of emerging ILTV strains could greatly

benefit from real time amplicon sequencing using the single-allele assay and MinION sequencing.

Isolation, identification and genomic characterizations of two different genotypes of avian paramyxoviruses 1 isolated from live bird markets in Tanzania in 2012

Iryna V. Goraichuk[a,b], Kiril M. Dimitrov[a], Peter L. M. Msoffe[c], Gaspar Honorati[d], Claudio L. Afonso[a], David L. Suarez[a]

[a]USDA-ARS-USNPRS-Southeast Poultry Research Laboratory, 934 College Station Rd., Athens, GA, 30605, USA.
[b]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, 83 Pushkinska St., Kharkiv, 61023, Ukraine.
[c]University of Dodoma, P.O. Box 259, Dodoma, Tanzania. d Tanzania Veterinary Laboratory Agency, P. O. Box 129, Mwanza, Tanzania.

Virulent strains of Newcastle disease virus *(*NDV*)* cause a devastating disease in poultry worldwide. Oropharyngeal and cloacal swabs were collected from chickens from live bird markets in four different regions of Tanzania, in 2012. Total RNA from swab-derived allantoic fluid was extracted using the QIAamp Viral RNA Mini kit. The Illumina libraries for next-generation sequencing were prepared using the Kapa Stranded RNA-Seq Library Preparation Kit. Pair-end sequencing (2×250 base pairs) of the generated libraries was performed on an Illumina MiSeq instrument using the 500 cycle MiSeq Reagent Kit v.2. Sequence data were assembled using MIRA software within a customized workflow on the Galaxy platform. All isolates were identified as virulent based on the cleavage site sequence and ICPI. The phylogenetic analysis of the complete genome sequences revealed that out of 10 isolates, 9 grouped together with isolates of genotype XIII and one with genotype V of NDV. Further phylogenetic and distance analyses based on the complete fusion gene revealed that within genotype XIII, all isolates belonged to genotype XIIIa, but separated into two sub-clusters (93.3% nucleotide identity), demonstrating the simultaneous circulation and evolution of at least two lineages of virulent sub-genotype XIIIa viruses in Tanzania. Within genotype V, the studied isolate clustered into sub-genotype Vd together with isolates from Kenya and Uganda (98.3 and 97.6% nucleotide identity). The obtained genomic sequences represent the first complete genome of velogenic NDV from sub-genotype Vd identified for the first time in Tanzania.

Expression and characterization of chicken anemia virus proteins as ELISA antigens

Linda O. Michel[a] and Daral J. Jackwood[a,b]

[a]Food Animal Health Research Program, [b]Department of Veterinary Preventive Medicine, The Ohio State University/OARDC, Wooster, OH 44691.

Chicken anemia virus (CAV), a member of the *Gyrovirus* genus in the family *Anelloviridae*, is a ubiquitous virus found in all poultry producing regions worldwide. CAV is an important avian pathogen that causes anemia and immunodeficiency leading to secondary infections. Diagnosis of CAV in chicken flocks and particularly breeder flocks is very important to the control of chicken anemia. The ELISA kits currently on the market use inactivated CAV antigens. The yields of virus can be variable and the inactivation process has the potential to reduce the quality of the antigens on the virus capsid. Replacing these antigens with baculovirus produced CAV antigens would provide ELISA kit manufacturers with a reliable high-quality source of antigen. Compared to chemical inactivation of live CAV, the use of heat to inactivate the baculovirus vector in such a CAV product does not damage the CAV epitopes. The CAV VP1, VP2 and VP3 genes from chickens were amplified with PCR and sequenced. The genes were cloned into plasmid transfer vector pVL1393 and recombined into the genome of baculovirus under the control of the polyhedron promotor. The proteins were expressed from baculovirus singly or in combination. Partially purified protein was then used to coat ELISA plates and an ELISA was performed using serum from CAV infected chickens.

A Retrospective Study (2016-2017) of Neoplastic Diseases Diagnosed in Backyard Chickens Submitted to the Alabama Department of Agriculture and Industries Veterinary Diagnostic Laboratory System

Erfan Chowdhury[a, b], John Roberts[b], Tami Kelly[a], Terry Slaten[a], Laurie McCall[a], Sara Rowe[a], Lanquing Li[a], Kellye Joiner[b] and Heather Walz[a]

[a]AL Department of Agriculture and Industries, Veterinary Diagnostic Laboratory system; [b]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

The records of Alabama Department of Agriculture and Industries, Veterinary Diagnostic Laboratory system (ADAI-VDL), from January 1, 2016 to December 31, 2017 were analyzed to determine the prevalence and type of neoplastic diseases that were diagnosed system wide. An overview of this study is presented here. All necropsies were performed according to the standard operating procedures of ADAI-VDL. In every case, a complete necropsy was performed, visible gross findings were identified, and samples were collected for further laboratory analysis. All neoplastic diseases were diagnosed by histopathologic observation. During 2016 to 2017 a total of 318 backyard poultry cases were submitted to the ADAI-VDL. Off those submission, neoplastic diseases were diagnosed in 105 (33%) cases. Among different neoplasms, Marek's disease (MD) was diagnosed in 85 (27%) and adenocarcinoma in 18 (5%) cases. Sarcoma, fibrosarcoma, leiomyosarcoma, and teratoma were diagnosed at 1 of each. In case of MD, lymphosarcoma was observed in multiple visceral organs with or without neural involvement. The adenocarcinoma was mostly ovarian origin, however in some cases the primary source could not be precisely determined. The finding of this study clearly demonstrates that neoplastic diseases are the most common cause of death in backyard chickens. Among various neoplasms, MD is the most prevalent. MD is highly controlled in commercial chickens due to vaccination but BY chickens are rarely vaccinated against this disease. Therefore, with the current increases in BY population there is an increase in susceptible chickens which can easily get infection through contact of infected chickens or contaminated environment.

In vivo testing of a qPCR for the specific detection of a new MDV serotype-1 vaccine (RN1250 strain) administered at day-old chicks

Andrea DELVECCHIO[1], Steve CRUSSARD[1], Stéphanie GALLEAU[1], Sylvain GOUTEBROZE[1], Peter BEHR[2], Jennifer HANEKE[2], Martin LIMAN[2], Michel BUBLOT[1], Stéphane LEMIERE[1]

[1] Boehringer Ingelheim Animal Health, Lyon, France; [2] ANICON, Hoeltinghausen, Germany

The CVI988 (or Rispens) strain of Gallid herpesvirus 2 commonly designated Marek's disease virus serotype 1 (MDV1) was developed in the Netherlands in the early 1970s and remains today the most efficacious MD licensed vaccine. The constant presence and the evolution of virulent MDV1 in the poultry population drive the need for research and improvement in the field of MD vaccination, especially for serotype 1 vaccine. Recently, a RN1250 Marek's disease vaccine strain containing a chimeric genome between different MDV strains was developed and proved to combine excellent safety and efficacy profiles. The objective of the present study was to evaluate the ability of a specific qPCR to detect the RN1250 in specific targeted organs and examine the vaccine virus replication profile in spleen, feather pulp, bursa of Fabricius and Thymus. Dust samples were also tested. The results showed that the qPCR technique was able to detect RN1250 replication in all the targeted organs of vaccinated chickens: the recovery of vaccine virus DNA was higher in the spleen, thymus and bursa of Fabricius than in feather pulps. The virus could also be detected transiently in dust. No virus could be detected in contact birds. RN1250 vaccine virus replication in target organs was observed to occur between 1 and 3 weeks post vaccination and the replication profile differ from the published replication of classical CVI988 vaccine.

Evaluation of an Easy and Rapid Detection of Avian Leukosis Virus Subgroup J (ALV-J) by Fully Automated POCKIT™ Central PCR System

Fu Choong Keat[a], Wei-Fen Tsai[b], Ping-Han Chung[b], Chia-Chi Chang[b], Yu-Chun Lin[b], Pin-Hsing Chou[b] , Simon Chung[b]

[a]Aviagen Inc. 920, Explorer Boulevard NW, 35806, Huntsville, AL, USA.
[b]GeneReach Biotechnology Corp., No. 19, Keyuan Second Road. Central Taiwan Science Park, Taichung, Taiwan, 407

ALV-J is a prevalent retrovirus that causes lympho-proliferative disease or tumor and results in serious economic losses in poultry breeding industries. The screening intensity is high especially in primary broiler breeding companies, commercial layer breeding, native chicken breeding, SPF (specific pathogen free) chicken last but not least biological products such as poultry vaccines. Contemporary available screening methods for ALV-J infection include RT-PCR, ELISA (antigen and antibody) and viral isolation. This evaluation and validation study of POCKIT™ Central Nucleic Acid Analyzer (POCKIT™ Central, GeneReach) were carried out in Asia region to compare with the available method such as RT-PCR and ELISA. An ALV-J RT-iiPCR reagent is commercially available to work with this system, with potential to facilitate timely identification of ALV-J based on the detection of env gene, which is usually used for discrimination of the subgroups. This study revealed the performance of the ALV-J RT-iiPCR/POCKIT™ Central system. It had analytical sensitivity of about 10 copies genome equivalents per reaction and did not cross react with ALV-A, -B, -E, and –K, and endogenous avian viruses (EAVs). Side-by-side comparison with a published real-time RTPCR system with 100 retrospective avian samples showed an interrater agreement of >90% in sensitivity and specificity. With analytical and clinical performance equivalent to the reference system, this user-friendly automated ALV-J RT-iiPCR/POCKIT™ Central system can serve as a sample-in-answer-out, minimize human error risks while providing easy qualitative results with cost effective reagents/consumables.

A Portable Microdevice for Size-based Virus Enrichment

Yin-Ting Yeh[A], Huaguang Lu[B], Mauricio Terrones[A]

[A]Department of Physics;
[B]Department of Veterinary and Biomedical Sciences The Pennsylvania State University, University Park, PA 16802

A size-tunable-enrichment-platform (STEP), our recently developed portable technology, is constructed by aligned and functionalized carbon nanotube (CNT) forests to enrich different viruses based on their sizes while removing major host contaminants of host cell debris, DNA, mRNA, etc. The CNT-STEP significantly improves detection limits and virus isolation rates up to 102 times. We have integrated the CNT-STEP with the next generation sequencing (NGS) technique to sequence unknown virus directly from field samples. Our preliminary results showed that our NGS viral reads increased from 2.9% (37,627 reads) to 90.6% (1,175,537 reads) after enrichment, indicating that the CNT-STEP could remove most of the non-viral contaminates from host samples. In testing a pooled duck cloacal swab sample, which was conformed positive to avian influenza virus (AIV) by virus isolation, the CNT-STEP was adjusted to 95 nm inter-tubular distance for AIV enrichment and concentration in the original duck swab sample. After enrichment, NGS and de novo sequence assembly yielded 8 AIV contigs in complete lengths, but no AIV related contigs were discovered in sequencing the duck swab sample without CNT-STEP enrichment. In our validation tests of H7N2 and H5N2 AIV samples, the CNT-STEP could make enrichment increases in two orders of magnitude of sequencing coverage that could dramatically enhance the sensitivity in identifying mutations. We continue working on for the establishment of a unique method CNT-STEP that enables close monitor of viral evolutions and a highly effective sample preparation platform to allow for efficiency in viral deep sequencing.

Isolation and TEM examination of unknown duck viruses

Lin Lin[1], Gang (Greg) Ning[2], Carrington Stephenson[1], Patricia Dunn[1], Huaguang Lu[1,3]

[1]Animal Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences
[2]Microscopy Core Facility, Huck Institutes of the Life Sciences
The Pennsylvania State University, University Park, PA 16802

In September 2018, a duck farm in Pennsylvania experienced unusual high mortalities of meat-type duck flocks at 5-6 weeks of age, over 100 deaths per week in a flock size of about 350 ducks. The dead ducks did not show typical clinical symptoms before they died, and necropsy exams did no see specific pathologic lesions. Tissue specimens of trachea, lung, heart, cecal tonsil and intestine collected from dead ducks were processed and inoculated into LMH cell cultures and also duck embryo cell cultures for isolations of possible duck viruses. After multiple cell passages, small rounding cytopathic effect (CPE) cells were observed. The CPE positive cell culture materials were tested negative for avian influenza virus, Newcastle disease virus, fowl adenovirus and avian reovirus. Then adequate amount (20-30 mL) of CPE positive cell cultures were produced for TEM examinations. Pellets of cell cultures and supernatant were processed for negative staining and pellets were also processed for embedding in resin for thin-sectioning. Negative staining showed clusters of virion particles in variety of sizes ranging from 35nm - 60 nm. A clear picture revealed the particles had a 35-40 nm core with capsules around it. Virion particles of ~30-40 nm were detected all over the thin-sectioned cell pellets. It appeared that the virions were probably detected in both nucleus and cytoplasm. Genome sequencing will be conducted for final identification of these duck virus isolations.

Validation of real time PCR reagents to identify Salmonella spp. DNA in enriched cultures

Jantina De Vylder[a], Gwenllyan Slacum[b]

[a]BioChek B.V. Fokkerstraat 14, 2811ER Reeuwijk, Netherlands.
[b]BioChek USA Corp. 3 Southgate Road, Scarborough, ME 04074

Salmonella spp. are one of the most common causes of food borne poisoning worldwide. Despite the implementation of extra hygienic measures and monitoring programs during the production life cycle of poultry, contaminated poultry products remain an important source of human salmonellosis. Detection usually involves time-consuming bacteriological methods. Rapid polymerase chain reaction assays, which are sensitive, reliable, and easy to use, have become more commonly used for routine screening of samples. BioChek has developed real-time PCR reagents for the identification of Salmonella spp. DNA in enriched cultures. Validation studies for the BioChek Salmonella Species PCR reagents used for the detection of *Salmonella* spp in environmental samples will be presented. The following criterial were evaluated: sensitivity, relative level of detection, inclusivity and exclusivity. The performance of the BioChek Salmonella Spp. PCR reagents was evaluated with over 500 samples by comparing with bacteriological methods.

A Comprehensive Comparison of multiple MG/MS Real Time PCR Assays

Heather Failyer

Georgia Poultry Laboratory Network

A non-biased, comprehensive comparison of multiple MG/MS PCR commercial kit manufacturers. There will be an in-depth look at sensitivity analysis between the kits using a various range of infection levels for commercial flocks from an independent testing facility. The pros and cons of each kit, as well as the ease of use and cost comparison will be evaluated.

Diagnostic Service for Avian Pathogenic *Escherichia coli* (APEC) Identification in Georgia: Comparison of Gene Profiles with Tissue of Isolation.

Nicolle L. Barbieri, Darby Newman, Lisa K. Nolan and Catherine M. Logue

Department of Population Health, PDRC, College of Veterinary Medicine, University of Georgia, Athens, GA

Colibacillosis caused by Avian Pathogenic *Escherichia coli* (APEC) is a significant cause of morbidity, mortality and carcass condemnation to the poultry industry worldwide resulting in significant economic losses. Here, we assess the use of a multiplex PCR panel for classifying APEC from diagnostic cases. A total of 66 isolates were used in this analysis collected between August and November of 2018 through PDRC diagnostic lab submissions. All isolates were screened using a multiplex PCR panel that targets genes associated with APEC chromosomal and plasmid virulence genes. Overall, isolates met the criteria for definition as well-developed pathogens with more than 90% of isolates being positive for the genes *iroN*, *ompTp*, and *hlyF*; 83% were positive for *aerJ* and 74% positive for *iss*; a significantly lower prevalence was observed for *cvaC*, *etsB*, *ireA* and *papC* (range 5-43%). APEC gene prevalence varied significantly with tissue of origin. The presence of any genes associated with the ColV virulence plasmid (*iss*, *iroN*, *hlyF, cvaC*, *etsB* and *ompTp*) were detected in 64 of 66 isolates (94%) suggesting that the plasmid which is considered a defining trait of APEC has become an established part of the host cell being required for virulence and other functions. The use of a multiplex panel to screen for APEC has shown good correlation with pathogenesis, and tissue source and correlates well with invasive strains. The path panel diagnostics is currently available through the PDRC diagnostic lab at University of Georgia and provides important information for APEC screening.

Next-generation random sequencing for identification and characterization of infectious agents in broilers with hypoglycemia and spiking mortality syndrome

Iryna V. Goraichuk[a,b], James F. Davis[c], Arun B. Kulkarni[c], David L. Suarez[a], Claudio L. Afonso[a]

[a]US National Poultry Research Center, Southeast Poultry Research Laboratory, 934 College Station Road., Athens, Georgia, 30605, USA.
[b]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, 83 Pushkinska St., Kharkiv, 61023, Ukraine. c Georgia Poultry Laboratory, 3235 Abit Massey Way, Gainesville, Georgia, 30501, USA.

Hypoglycemia and spiking mortality syndrome (H-SMS) is a malady of broilers chicks characterized by sudden increase in mortality. The affected birds are hypoglycemic, with blood glucose levels of less than 180 mg/dl. Clinical signs may include tremors, ataxia, and blindness. The etiology of H-SMS is unknown; however, the successful experimental reproduction of H-SMS with the use of homogenized organs, indicates that an infectious agent must be involved. Previous investigations have indicated an association of spiking mortality with reoviruses, adenoviruses or arenavirus-like agents. In order to identify the infectious agent(s) of H-SMS, in different field and experimental samples clinical cellular extracts from pancreas or inoculated embryos were analyzed using next-generation sequencing. Total nucleic acids from homogenized tissues were extracted using the Qiagen DNeasy Blood and Tissue Kit. RNA was reverse transcribed and randomly amplified and cDNA or DNA Illumina libraries for next-generation sequencing were prepared using the the Nextera XT DNA library preparation kit. Fragment size distribution and concentration of the DNA libraries were checked on a Bioanalyzer 2100 using the Agilent High Sensitivity DNA Kit. Pair-end sequencing (2x250 base pairs) of the generated libraries was performed on an Illumina MiSeq instrument using the 500 cycle MiSeq Reagent Kit v2. Sequence data were assembled using MIRA software within a customized workflow on the Galaxy platform. Sequence reads were analyzed by the Kraken

Metagenomics classifier on the Galaxy platform and distinct infectious agents were detected from different field cases. Metagenomics results were verified by real time PCR, regular PCR, reference genome assembly, or Sanger sequencing of the specific amplicons.

Why a Quality Management System is Valuable in A Poultry Library

Brenda Glidewell

GA Poultry Lab

The implementation of a Quality Management System (QMS) within a Poultry Diagnostic, Monitoring, or Research Laboratory provides value not only to the customers but to the employees, vendors, State, and Federal Government. Within the QMS the guidelines used ensure quality results are reported in a timely and efficient manner to the customer; that kits, reagents, equipment and supplies are validated as "best fit" for the services being offered; and that there is confidentiality of results. The QMS requires that equipment be maintained and calibrated on a regular basis to ensure accurate and precise test results. The ongoing training program ensures that technical employees are authorized and trained to run specific assays and that they have passed proficiency tests for the assays they run.

Enteric Health

The effect of dietary supplementation of several Bacillus strains on growth performance and gut health in mixed coccidiosis infection in chickens

Atul A. Chaudhari, Youngsub Lee, Hyun S. Lillehoj

Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, ARS, U.S. Department of Agriculture, Beltsville, MD 20705, USA.

Present study investigated the effects of dietary supplementation of several Bacillus strains on growth performance, intestinal inflammatory and anti-inflammatory cytokines, antioxidants and tight junction (TJ) protein mRNA expression in chickens challenged with mixed coccidia infection (Eimeria tenella, E. maxima and E. acevirulina). Firstly, ten different strains were screened for their beneficiary effect after coccidia challenge by measuring relative body weight gain (RBWG), lesion score and total oocyst count. Secondly, three Bacillus strains were evaluated in depth by measuring RBWG, lesion score, total oocyst count and gene expression of proinflammatory (IL-6 and IL-8), anti-inflammatory (IL-10 and TGF-ß), anti-oxidants (SOD and HMOX) and tight junction proteins (JAM 2 and occludin). Our results showed that out of ten different strains three strains, one B. licheniformis and two B. amyloliquifacien fed birds showed significant RBWG, low lesion score (caeca, jejunum and duodenum) and lower oocyst count compared to non- Bacillus-fed control birds. Bacillus fed birds showed significant pro and anti-inflammatory response and higher expression of antioxidants and tight junction proteins in duodenum, caeca and jejunum. In conclusion, present study results are promising and indicate beneficiary effect of probiotic supplementation in poultry diet to control economic losses imposed by coccidia infection in chickens.

Food Safety

The Effectiveness of a Feed Grade Sodium Formate in Feed or Drinking Water in Reducing *Salmonella heidelberg* Colonization of Broilers

Charles Hofacre,[†] Joshua Jendza,[*] and Roy Berghaus[††]

[†]Southern Poultry Research Group, Watkinsville, GA
[*]BASF Animal Nutrition, Florham Park, NJ
[††]The University of Georgia, Athens, GA

A study to evaluate the effectiveness of a feed grade sodium formate (formate) product (0.60%) fed continuously from day 1 to 42 or day 35 to 42 or in the birds drinking water day 37 to 42 versus a sodium bisulfate (bisulfate) product in the drinking water day 37 to 42 with eight replicates per treatment. One-half of chicks in each pen were

tagged and challenged (Day 1) with a nalidixic acid resistant *S. heidelberg*. At 42 days after feed withdrawal, five ceca of challenged (direct) and ten ceca of penmates (horizontal challenged) were aseptically removed. Prevalence and enumeration were performed by micro most probable number (MPN) method of Berghaus et al., 2013 with 25 µg/ml nalidixic acid in XLT-4. Crop pH was also measured. The continuous fed formate had a significant reduction in ceca S.H. prevalence (46.7%a), followed by feed formate at 35-42 (49.2%ab), water formate (62.5%ab) and water bisulfate (75%b). All of the formate treatments in the feed had a numerical reduction in S.H. MPN/g with the d1-42 having the greatest reduction. Using a Tobit regression model to more closely estimate the ceca Salmonella MPN status (censoring culture negative at -0.5log10 MPN/g) demonstrated that continuous fed formate significantly lowered the estimated mean log10 MPN/g (-0.79a), followed by d35-42 formate (-0.69ab), water formate (-0.15ab) and water bisulfate (0.11b). In conclusion, use of the feed grade sodium formate in the feed or drinking water of broilers can significantly decrease the prevalence and number of S.H. positive ceca.

Estimating Salmonella Numbers to the Processing Plant: a Correlation of *Salmonella* Enumeration of Bootsocks, Ceca and Carcass Rinses from Broiler Chickens

Virginia Baxter[1], Charles L. Hofacre[1], Roy D. Berghaus[2], Kalen Cookson[3], and Manuel Da Costa[3]

[1]Southern Poultry Research Group
[2]The University of Georgia
[3]Zoetis Global Poultry

Understanding the load of *Salmonella* coming to the processing plant will aid in determining how well pre-harvest interventions are working. An on-farm study in 2013 by Berghaus et al. found that *Salmonella* prevalence and load of bootsocks correlated to the *Salmonella* load in whole bird rinses with feathers on. Two controlled pen studies were performed where birds were sampled to evaluate the correlation of prevalence and load of *Salmonella* bootsocks, ceca and carcass rinse samples. *Salmonella heidelberg* in study #1 and *S. kentucky* in study #2 were given to 50% of the birds in each pen at four days of age. Ten horizontally challenged birds per pen were processed at a small-scale pilot processing plant at 42 days of age. After processing, carcass rinses and ceca (post-rinse) were collected from the ten horizontally challenged birds. Additional ceca were taken from five directly challenged birds per pen, and bootsocks were collected from each pen. The data from this study was used to evaluate if the bootsock Salmonella MPN and prevalence could be used as a predictor of ceca MPN and carcass rinse MPN. In study #1, the relationship between bootsock MPN and carcass rinse MPN was not statistically significant, however there was a statistically significant positive association between bootsock MPNs and pen-level mean ceca MPNs and prevalances.

Salmonella enterica subsp. *enterica* serovar typhimurium weakens avian blood macrophage-like monocytes functions *in vitro*

Paniz Zarghami[1], Elya Abbaszadeh[2], Sahar Rostami[1], Mostafa Peyghambari[2], Jalil Mehrzad[1], Ramak Yahyaraeyat[1], Taghi Zahraei Salehi[1]

[1]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[2]Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Salmonella enterica serovar typhimurium is one of the most important pathogenic *Salmonella* species in human and animals which causes substantial economic losses in poultry industry. Monocytes and macrophages have important role in the killing of intracellular pathogens such as *Salmonella*. The ability of *Salmonella enterica* to survival in these innate immune cells is one of their virulence factors. The aim of this study is to investigate the effect of *Salmonella enterica* serovar typhimurium on the functions of avian blood macrophage-like monocytes *in vitro* and provide the appropriate experimental models on cell culture and challenge of avian phagocyte cells to *Salmonella,* which provide more insight on the cellular and molecular

aspects of host-pathogen interactions in avian species. Homogeneous macrophage-like monocytes were isolated and cultured from peripheral blood mononuclear cells of 2-3 weeks-old broilers. After quantitative and qualitative evaluation of macrophage-like monocytes by microscopy and flow cytometry, the cells were challenged with *Salmonella enterica* serovar typhimurium. Afterwards, the mRNA expression of IL-1β in all groups of macrophage-like monocytes were assessed with PCR and qPCR method. Results shows that *Salmonella enterica* serovar typhimurium weakens and decreased mRNA expression of IL-1β. We are currently further working on how such a down regulating effect on mRNA of key pro-inflammatory cytokine, IL-1β, classically/mechanistically occur in innate immune cells and molecules. This finding opens new window for researchers to conduct more research on the molecular aspect of *Salmonella*- macrophage interactions and pathogenicity of salmonellosis and other bacterial diseases in animals especially avian species.

Salmonella Field Isolates: A Retrospective Analysis of Salmonella Serotypes Association with Common Field Isolation Media of Poultry Flocks in the Southern United States

[1]Carolyn Miller, [2]Gordon Whitbeck, [3]Jessica K. Hockaday

[1]Consultant, [2]Whitbeck Laboratories, [3]Aviagen, North America

The goal is to determine if an association of Salmonella serotypes or serogroups have significant affinity to specific environmental media with poultry houses. A retrospective data analysis will be conducted with Salmonella isolates submitted from broiler, broiler-breeders, laying hens and primary broiler-breeder flocks from the Southeastern US as the initial data set.

What to do with that Salmonella culture over the weekend?

Doug Waltman

Bacteriology Technical Director, Georgia Poultry Laboratory Network, 3235 Abit Massey Way, Gainesville, GA, 30507

The isolation procedure for Salmonella is a multi-step process involving primary enrichment, secondary enrichment and plating. Each step requires incubation for 24 hours. There are no logistical laboratory problems if samples are set up on Monday or Tuesday, however if samples are set up on Wednesday, Thursday, or Friday, they will need to be transferred by someone on Saturday and Sunday or a deviation from the standard protocol will occur. The question is often asked “what can I do with my samples, leave in the incubator, put on counter or put in refrigerator”? We began a project to determine the best method of “holding over” samples because of a weekend or long holiday. We used routinely submitted samples and incubated primary and secondary enriched samples and then held them for 3 days either at incubation temperature, at room temperature or in the refrigerator and processed them each day. We also did the same study with plated samples. Results will be presented as well as recommendations for Salmonella sample holding.

Immunology

Development of Sandwich ELISA for Differential Detection of Chicken Heterodimeric Cytokines, Interleukin-12 and -23

Woohyun Kim, Hyun S. Lillehoj

Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, United States Department of Agriculture

Interleukin (IL) -12 cytokine family consists of IL-12, IL-23, IL-27 and IL-35. Unlike mammals, there are

only two cytokines have been identified in chickens so far among the IL-12 family, IL-12 and IL-23. They are heterodimeric cytokines consisting of two covalently linked subunits, IL-12; p35 (IL-12a) and p40 (IL-12/IL-23ß) and IL-23; p19 (IL-23a) and p40. Since p40 subunit is shared between IL-12 and IL-23, it is difficult to identify the nature of cytokine if PCR-based detection method using p40 is used to measure IL-12 and IL-23 production. In the present study, we developed mouse monoclonal antibodies (mAbs) which are specific against three subunits, p19, p35 and p40, and developed the sandwich ELISA to differentiate IL-12 and IL-23 expressions. The p40 mAb was used as capture antibody and p35 or p19 mAb was used as detection antibody for IL-12 and IL-23 differentiation, respectively. Some of the new mABs we developed also neutralized the function of IL-12 and IL-23 and blocked production of IFN-? and IL-17A in chicken T cells. In summary, we have generated novel sensitive detection system to differentially identify chicken IL-12 and IL-23 at the level of protein expression. This method will be valuable to obtain an insight to understand chicken cell-mediated immunity.

Development of novel monoclonal antibodies against chicken interleukin- 4 and alternative activation of macrophage-like cells in chickens

Atul A. Chaudharia, Woo H. Kim[a], Hyun S. Lillehoj[a]*

[a]Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, ARS, U.S. Department of Agriculture, Beltsville, MD 20705, USA.

The functions of a Th2 cytokine such as interleukin 4 (IL-4) in chickens is not well understood due to the lack of specific immune reagents against chicken IL-4 (chIL-4). In mammals, IL-4 stimulate alternative activation of macrophages. In the present study, we developed mouse monoclonal antibodies (mAbs) against chIL-4 and studied alternative activation of macrophage. Alternative activation of HD11 cells was investigated by measuring nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) expression, arginase activity and gene expressions of *iNOS*, *CD80* and *CD86* (M1 markers) and chemokine (C-C motif) ligand 17 (*ccl17*) and mannose receptor C-type1 (*MRC1L-A*) (M2 markers) in HD11 cells treated with chIL-4, lipopolysaccharide (LPS), chIL-4+LPS, chIL-4+LPS+mAbs. The mAbs successfully detected the endogenously produced chIL-4 (chicken sera, cell supernatant and pellet) by capture ELISA, ICC and flow cytometry. Further, NO production by LPS-stimulated HD11 cells and primary monocyte/macrophage cells was inhibited by chIL-4 with reduced iNOS expression and increased arginase activity. Also, chIL-4 induced robust expression of M2 marker genes than M1-related genes. All these effects were neutralized by anti-chIL-4 antibodies. In conclusion, our results showed that newly developed anti-chIL-4 mAbs could serve as valuable immune reagents to detect chIL-4 and demonstrated that chIL-4 may regulate alternative activation of HD11 cells in chicken.

Development of Poultry-Specific Immune Reagents

Hyun Lillehoj, Woohyun Kim, Charles Li, Zhifeng Sun

Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, ARS, U.S. Department of Agriculture, Beltsville, MD

The goals of this NIFA-funded grant are 1) to identify chicken immune molecules, particularly cytokines, chemokines and cell surface markers, express them as recombinant proteins, and characterize their function, and 2) to develop monoclonal antibodies (mAbs) to the target chicken molecules. Cloning of chicken genes (20 in total) were carried out the number of sets of primers and synthesized to amplify based on the chicken genomic and mRNA sequence. The recombinant proteins were obtained from E. coli, mammalian cells or yeast. Hybridomas were developed, specificity of mAbs were validated using several assays including ELISA, immunohistochemistry, Western blot, flow cytometry, qPCR, cell proliferation, and nitric oxide assay. All the target we selected have shown to have critical functions in host defense against pathogens and all recombinant proteins expressed have met the quality standard. In summary, we have expressed 20 proteins (11 from yeast, 9 from E. coli) and 5 proteins expressed from mammalian system

for mAb development and functional study. Twenty target proteins consist of 13 cytokines, 4 chemokines, 1 surface receptor, and others. For mAb development, the progress is at various stages with 3 finished, 9 in characterization, 4 in production, and 2 in screening. The mAbs developed in this study represent new sets of immune reagents which are species-specific for poultry.

Clostridium perfringens induces IL-10 secretion in chicken intestinal epithelial cells and necrotic enteritis broiler chicken

Youngsub Lee, Woohyun Kim, Hyun S. Lillehoj

Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, ARS, U.S. Department of Agriculture, Beltsville, MD

The Aim of this study was to assess the production level of interleukin (IL)-10 in Clostridium perfringens (CP)-stimulated intestinal epithelial cells (IECs) and CP-infected chickens using an antigen capture ELISA developed by mouse monoclonal antibodies against chicken IL-10. Chicken IECs were stimulated with CP toxin in the absence or presence of antibodies (a-toxin or NetB) for 18?h. Expression of chicken IL-10 and IL-6 was monitored by quantitative real-time polymerase chain reaction. Serum and jejunum samples were collected from CP-infected chickens at 9 days post-infection and expression of IL-10 and IL-6 was analyzed. IL-10 production was detected by antigen capture ELISA in chicken IECs stimulated with CP and in serum samples collected from CP-infected birds. The mRNA levels were consistent with the results of antigen capture ELISA. CP induced IL-10 production in chicken IECs. Increased IL-10 production was detected in CP-stimulated chicken IECs and in serum collected from CP-infected birds, using antigen capture ELISA. in conclusion, antigen capture ELISA could be a useful tool to monitor IL-10 production in chicken disease.

Comparison of toll-like receptor (TLR) 3 mediated immune responses in wild-type and TLR3 knockout chicken and quail fibroblast cell lines

Mahesh K C,[1, 2] John M. Ngunjiri,[2] Amir Ghorbani,[1, 2] Mohamed Elaish,[2] Michael Abundo,[1, 2] Kichoon Lee,[3] Chang-Won Lee[1, 2, *]

[1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University;
[2]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University;
[3]Department of Animal Sciences, College of Food, Agricultural, and Environmental Sciences, The Ohio State University

Chicken and quail differ in TLR3 expression, where only quail TLR3 undergo alternative splicing and generate two additional isoforms. To understand the TLR3 mediated immune response among avian species, TLR3 knockout (KO) chicken (DF1) and quail (QT35) fibroblast cell lines were generated by a CRISPR/Cas9 system optimized for avian species. The wild-type (WT) and KO cells were compared for replication of low pathogenic avian influenza virus. We observed no difference in virus replication in QT35 WT and KO, but 10-fold decrease in KO DF1 cells compared to WT cells. The differences in virus replication between DF1 and QT35 cells may reflect differences in TLR3 mediated immune responses. To measure immune responses, the cells were treated with poly(I:C) to quantify IFNβ and IL-8 mRNA expression and type-I IFN induction using a IFN-sensitive vesicular stomatitis virus-based bioassay. In WT and KO QT35 cells, poly(I:C) was able to induce biologically active type-I IFN and there was no difference in IFNβ mRNA levels. However, a significantly high IL-8 mRNA expression level was observed in WT compared to KO cells. Attempts to compare immune responses in WT and KO DF1 cells was unsuccessful due to induction of early apoptosis in the WT which was not observed in KO cells regardless of different concentrations of poly(I:C). Together, these data suggest species-specific roles of TLR3 in mediating immune responses. Our ongoing studies will further delineate the species-specific roles of TLR3 together with other dsRNA

recognition receptors like MDA5 in mediating immune responses in relation to influenza infection.

Expression and Characterization of Chicken Perforin and Granzyme A

Charles Li, Zhifeng Sun, Woohyun Kim, Hyun Lillehoj, Hongyan Zhao, and Mingmin Lu

Animal Bioscience and Biotechnology Laboratory, Beltsville Agriculture Research Center, Agricultural Research Service, USDA, Beltsville, MD 20705

Perforin and granzymes are pore forming cytolytic proteins and serine proteases, respectively, found in the granules of cytotoxic T lymphocytes and Natural Killer cells, and are important players in immune responses. Upon degranulation, perforin binds to the target cell's plasma membrane, and form pores on the target cell, allowing for the passive release of granzymes from endolysosomal vesicles into the target cells. Granzyme B does not seem to exist in chickens. Granzyme A is one of the most biologically important granzymes which induce programmed cell death (apoptosis) in the target cells, thus eliminating cells that have become cancerous or are infected with viral or bacterial pathogens. In this study, chicken perforin (ckPRF1) and granzyme A (ckGZMA) proteins were expressed and characterized. A 2478-bp extracellular region of ckPRF1 gene was cloned in pET28a(+) vector and expressed in BL21-AI™ *E. coli.* The recombinant ckPRF1 proteins were around 46kDa, 25kDa and 16 kDa, and cross-reacted with monoclonal antibody (mAb) specific for human PRF1 (Clone F-1). A 711 bp extracellular region of ckGZMA gene was cloned in pRSET-C expression vector. The recombinant plasmids were transformed into *E. coli* strain BL21 Star (DE3) (pLysS) cells. The expressed ckGZMA protein was around 37 kDa, and cross-reacted with a mAb specific for human GZMA (Clone 3G8.5). These proteins will be used for mAb development which will be helpful for the detection of these proteins in biological samples. Availability of antibodies for immune molecules will greatly benefit the avian immunology community and promote poultry health and production.

Effect of in ovo vaccination with HVT on IFN-γ and TLR-3 transcripts in the spleen and lung of chicken embryos

A. Boone [1,2,] A.L. Cortes[1], F. Perozo[3], S. Lemiere[3], and I.M. Gimeno[1]

[1]North Carolina State University, College of Veterinary Medicine, Raleigh, NC
[2]Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC
[3]Boehringer Ingelheim Animal Health

In ovo vaccination with HVT hastens immunocompetence of chickens. Initially we demonstrated this positive effect of HVT in SPAFAS chickens and more recently we have shown a somewhat similar response in commercial meat type chickens. Although the mechanisms behind this effect are not fully understood, a chronological evaluation of interferons (IFN), IFN receptors, and toll-like receptors (TLR) in SPAFAS embryos following HVT vaccination at 18 days of embryonation (ED) showed an increase in IFN-γ and TLR-3 transcripts in the spleen and lung. The objective of the present study was to evaluate if IFN-γ and TLR-3 transcripts are also increased in meat type chicken embryos after HVT vaccination. Two groups of embryonated chickens were vaccinated at 18 ED with the recommended dose of a commercial HVT (6,080 PFU) via intra amniotic or were sham inoculated with the vaccine diluent. Spleens and lungs were collected at hatch and IFN-γ and TLR-3 transcripts were evaluated by q-RT-PCR. Results will be discussed.

Identification of nucleotide-binding oligomerization domain like receptor pyrin domain containing 3 inflammasome in chickens

Ching Ching Wu, Chun-Yu Tung, and Tsang Long Lin

Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907

Studies were undertaken to identify chicken innate immune cytosolic sensor nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3 (NLRP3) inflammasome. RNA was

extracted from chicken macrophage cell line (HD11 cells) or bursae, subjected to PCR amplification of chicken NLRP3, and followed by cloning and sequencing. Functional characterization of NLRP3 was determined by 12-hour lipopolysaccharide (LPS) and additional 15-minute ATP stimulation of HD11 cells. Chicken IL-1ß levels in the cell culture supernatants were then analyzed by Western Blotting. The chicken NLRP3 has an open reading frame encompassing 2778 base pairs of nucleotides and encoding a protein of 925 amino acids. There is one pyrin domain (PYD) in the N-terminal region and leucine-rich repeat domain (LRR) in C-terminal region. Production of mature chicken IL-1ß was detected on the Western blots from the supernatants of HD11 cells stimulated with LPS for 12 hours. Increased amounts of chicken IL-1ß were detected in the supernatants of HD11 cells, following stimulation with LPS for 12 hours and exposure to ATP for additional 15 minutes. The results indicated that chicken tissues possess NLRP3 inflammasome, encompassing 2778 base pairs of nucleotides and encoding for 925 amino acids. Chicken NLRP3 functions as a cytosolic sensor for LPS and ATP and production and activation of mature chicken IL-1ß is chicken NLRP3 dependent.

Development of The Immune System In Broiler Breeders: Impact Of IBDV Vaccination and Feeding Programs

Enrique Montiel

Boehringer Ingelheim

The development of the main immune organs in broiler breeders pullets reared under various feeding programs in controlled or field conditions was studied. Day-old broiler breeders were *in ovo*-vaccinated with a vector HVT + Infectious bursal disease (IBD) vaccine (VAXXITEK®) + SB1 (Boehringer Ingelheim, Gainesville, GA). Two groups were placed in an experimental broiler breeder facility at the University of Georgia. A third group was placed in a commercial pullet farm in Northeast Georgia on re-used litter. All birds were fed *ad libitum* for 2 weeks and started on skip-a-day (SAD) or everyday in the feeder (EDF) feeding programs for the duration of the study. At 2, 3, 4, 5, 7, 12 and 17 weeks of age, spleen, bursa and body weights were recorded from 5 birds per group and tissue samples were collected in 10% buffered formalin for histopathological analysis. Bursal weights were numerically higher in the EDF group at 3, 4, 5, 7, 12 and 17 weeks, No significant differences among the spleen weights of the three groups (P=0.94) were observed. Histopathology results revealed virtually no bursal lesions up to 17 weeks of age in any of the groups. Thymus histopathology showed minimal lesions at 4, 7, 12 and 17 weeks of age in all groups. The number of spleen germinal centers (GC) ranked from 1.2 to 34.8 GC/tissue section throughout the sampling period and there were no significant differences (P>0.05) among the various groups. These results differ from conventional knowledge about bursal health in broiler breeders.

CORRELATION BETWEEN THE LEVEL OF MATERNAL ANTIBODIES IN BROILERS AGAINST CHICKEN ANEMIA VIRUS AND INFECTIOUS BURSAL DISEASE VIRUS WITH RESPECT TO THE TIME OF EGG STORAGE AND THE AGE OF THE BREEDERS

Roa-Garcia XA[1], Cortes-Cuevas A[2], Rojo-Barrañón FJ[1], Gómez-Verduzco Gabriela[2]

[1] Boehringer Ingelheim Animal Health Mexico
[2] National University Autonomos of Mexico

In order to evaluate the relation between the maternal antibodies levels in 1 day old chicks against Chicken anemia virus (CAV) and infectious bursal disease virus (IBDV) from breeder with different ages (54, 44 and 36 weeks) at different egg storage periods (5, 10 and 14 days); an experimental design with a 3 X 3 factorial arrangement was used. An Effect of age factor of the breeder was observed ($P < 0.05$) showing higher antibodies titers against CAV in birds from breeders with 44 and 54 weeks old compared to those of 36. There was also an effect ($P < 0.001$) on the storage period of the egg with the highest antibodies titers against CAV in the period of 5 days followed by the periods of 10 and 14. Regarding antibodies titers against IBDV, higher titers ($P < 0.001$) were observed in chicks from older breeders. The storage period effect was observed showing lower titers ($P<0.001$) against IBDV as the storage period advanced (5, 10 and 14 days).

To concluded, age of the breeders is related to the increase of the antibodies titers in the chicks. The egg storage period decrease antibodies titers in the chicks for CAV and IBDV.

Comparison of the Serological Response of Three Tetravalent Commercial Inactivated Vaccines (ND, IB, IBD and REO) in Breeders

Bruno Garcia[1], Eliana Icochea[1]

[1]School of Veterinary Medicine, San Marcos University

It was compared the serological response induced for three commercial tetravalent inactivated vaccines against Newcastle disease (ND), infectious bronchitis (IB), bursal infection (IBD) and Reovirus (REO) in broiler breeders. For this purpose, were used 37149 female broiler breeders 1-day-old, Cobb Vantres 500, divided in three groups of 12,402, 12,389 and 12,358 each one (Groups 01, 02 and 03). During the grow out stage, the birds received the same vaccination program with live vaccines against the four agents. At 20 weeks of age the birds of each groups were vaccinated with three different commercial tetravalent inactivated vaccines. At 25, 30, 35, 40, 45, 50, 55 and 60 weeks, were collected 20 blood samples per group to be analyzed by ELISA tests using a kit of the IDEXX Laboratories. Antibody titers were described as geometric means per flock and comparisons between the geometric means of antibody titers between experimental groups were analyzed by ANOVA and multiple comparisons of Duncan. Significant statistical differences between most groups was observed. Only no significant differences were found in the antibody titers for ND at 50 weeks between groups 1 and 2 and, in the antibody titers for REO at 45 weeks between groups 2 and 3, indicating that at these ages and with these agents, the serological response was very similar.

Effects of glycinated zinc on host immune response to necrotic enteritis in broilers

Theros Ng

University of Georgia

This experiment studied the effects of glycinated zinc (B-TRAXIM®2C-Zn, Pancosma, CH) on broilers infected with necrotic enteritis (NE). Broiler chicks were randomly distributed into four treatments with six replications each: uninfected group fed 40 mg/kg of glycinated zinc and infected groups fed 40, 80, or 120 mg/kg of glycinated zinc. Chickens were infected with 5,000 *Eimeria maxima* oocysts per bird on d14 and 108 CFU *Clostridium perfringens* on d19, 20, and 21 by oral gavage. Feed and water were given *ad libitum*. On d21, three bird/pens were euthanized for lesion scoring and sample collection. The remaining birds were euthanized on day 35 to evaluate growth performance during the recovery phase. Average daily gain, average daily intake, and anti-*Clostridium perfringens* IgG in serum and IgA in bile were not different on day 21
($P>0.05$). NE infection increased feed conversion ratio by 18.0% on d21 ($P<0.05$) and by 21.6% on d28 ($P<0.05$). Uninfected birds had the lowest intestinal lesion scores ($P<0.01$), infected birds fed 40 and 80 mg/kg of zinc had the highest lesion scores ($P<0.01$), and infected birds fed 120 mg/kg of zinc had lower lesion scores than birds fed 40 & 80 mg/kg zinc. Pro-inflammatory and anti-inflammatory cytokines, tight junction proteins, and zinc transporter and anti-oxidant proteins mRNA gene expressions will be analyzed in jejunum, cecal tonsils, spleen, and liver tissues. Inclusion of zinc above 40 mg/kg from glycinated zinc improved production performance from NE infection; but doses of 120 mg/kg of zinc was needed to alleviate from intestinal damages.

Marek's Disease Virus

The effects of CVI988 Marek's disease vaccine in eliciting transcription of IFN- and TLR-3 in turkey embryos

J. Bremer, A.L. Cortes, E. Gonder, A. Turner, I.M. Gimeno

North Carolina State University

The use of recombinant vector vaccines has expanded in the poultry industry. Marek's disease (MD) vaccine virus (i.e. HVT) has been widely used as a vector since it induces extended protection, can be administered in ovo, and does not interfere with maternal antibodies. In addition to HVT, serotype 1 MD vaccine CVI988, has been used as vector in experimental vaccines with high levels of success. In the turkey industry, it is becoming common to use a recombinant HVT (rHVT) carrying inserts of Newcastle disease virus (rHVT-ND). In previous work, we have demonstrated that rHVT-ND should be administered in ovo to prevent interference with wild type HVT in the first week of life. Furthermore, the study showed that both rHVT-ND as well as CVI988 replicated readily in the turkey embryo. Even though replication of rHVT-ND was high, we could not demonstrate that this vaccine activated TLR-3 and IFN- in the turkey embryos as we had described previously in chicken embryos. We hypothesized that since the turkey is the natural host for HVT, this may affect the immune response that the bird elicits. The purpose of this study was to evaluate the effect of a chicken-origin vaccine CVI988, administered in ovo, on the activation of TLR-3 and IFN- in the turkey embryo. Results will be discussed.

Effect of challenge with oncogenic Marek's disease virus on the replication of Marek's disease vaccines

Rachel Reese, Aneg L. Cortes, Maria Correa, Gustavo Machado, Isabel M. Gimeno

North Carolina State University College of Veterinary Medicine

In the last decade, monitoring Marek's disease (MD) vaccination by real time PCR has become a common practice. Evaluating in vivo replication of MD vaccines in the feather pulp at 7-10 days of age provides information on how well a flock has been vaccinated. Factors such as vaccine dose, combination with other vaccines, age and route of vaccination, and the origin of the vaccine can influence the results and need to be taken into consideration. Early infection with oncogenic Marek's disease virus (MDV) could also affect how vaccines replicate in the first week and therefore might influence the results. The objective of this study was to evaluate if coinfection with oncogenic MDV could affect MD vaccine replication. A retrospective study was done using data from seven animal experiments in which chickens were vaccinated against MD either in ovo or at day of age and challenged with various MDV at day of age by contact. In each experiment, vaccinated but not challenged groups were used as control. Replication of MD vaccine was evaluated in samples of feather pulp collected at 7-10 days of age by real time PCR. Statistical analysis was conducted to evaluate the effect of oncogenic MDV on MD vaccine replication. Results will be discussed.

Study of the efficacy and replication of recombinant vector vaccines using herpesvirus of turkey (rHVT) when combined with other Marek's disease vaccines

A.L. Cortes, I.M. Gimeno, A. Reilley, T. Barbosa, I. Alvarado, R. Koopman, A. Martinez.

North Carolina State University

Several recombinant HVTs (rHVTs) have been developed within the last decades and they are commercially used worldwide. While in broiler chickens, rHVTs are usually administered alone, in long-living birds they are used in combination with Marek's disease (MD) vaccines of other serotypes (i.e. CVI988). The objectives of this work were (1) to evaluate protection against MD conferred by HVT and two rHVTs when combined with CVI988 and (2) to optimize the use of rHVT in combination with CVI988 to maximize replication of rHVT without compromising MD protection. Various vaccine protocols, all using rHVT or HVT at the recommended dose (RD), were evaluated. They included in ovo vaccination with HVT+CVI988 or rHVT+CVI988 (using either double dose, DD, or the standard recommended dose, SRD, of CVI988), day of age vaccination of rHVT+CVI988 at DD, and revaccination protocols using rHVT in ovo followed by CVI988 at DD at day of age. Our results show that, when combined with CVI988, HVT and rHVTs confer similar level of protection against MD (above 90%) regardless if CVI988 was used as the SRD or at DD. However, combination of rHVT with CVI988 at DD resulted in reduced replication rates of rHVT (60-76% vs 95-100%) which might have an effect on the expression of the insert. Our results show that such negative effect could be avoided without jeopardizing MD protection by administering CVI988 at SRD (if combined in ovo with rHVT) or administered rHVT first in ovo followed by CVI988 at DD at day of age.

Role of Meq-vIL8 in the pathogenesis of Marek's disease virus

Kanika Bajwa, Yifei Liao, Mohammad AI-Mahmood, Sanjay M. Reddy and Blanca Lupiani

Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, 77843, USA

Marek's disease virus (MDV) is a highly contagious alphaherpesvirus that causes rapid onset of lymphoma and paralysis in chicken. The MDV genome encodes for an oncogene, Meq, a chemoattractant of chicken peripheral blood mononuclear cells, vIL8 and a splice variant between meq and vIL8 (meq-vIL8). Chickens infected with a recombinant virus lacking the *meq* do not develop tumors, while chickens infected with a mutant virus lacking *vIL-8* show severely
impaired replication in B-cells and tumor development. However, it is unclear whether these phenotypes are due to loss of Meq and vIL8 individually, or because of the splice variant Meq-vIL-8. To specifically examine the role of MeqvIL-8 in MDV pathogenesis, we constructed two recombinant viruses: MDV/vIL8ST (where the introns of *vIL8* were deleted) and MDV/mutmeq (where the splice donor site of *meq* was mutated) from a very virulent plus strain of MDV (686). RT-PCR and qPCR analysis show that MDV/vIL8ST and MDV/mutmeq lack the *meq-vIL8* splice variant. In addition, the mutation of *meq* splice donor site did not affect viral replication *in vitro* or *in vivo*, which was confirmed by growth kinetics and by analyzing genomic copy numbers. No changes in transformation efficiency were observed following analysis of gross lesions and tumor formation compared to parental MDV virus. Interestingly, the MDV/mutmeq resulted in significantly shortened mean death time. To the best of our knowledge, our study is the first to reveal the role of Meq-vIL8 splice variant in MDV pathogenesis.

Parasitology

Fenbendazole Resistance in the Turkey Nematode, *Ascaridia dissimilis*

James Collins[1]*, Brian Jordan[2], Luke Baldwin[3], Claude Hebron[4], Kelsey Paras[1], Ray Kaplan[1]

[1.] University of Georgia College of Veterinary Medicine Department of Infectious Disease Athens, GA 30602
[2.] University of Georgia College of Agriculture and Environmental Sciences Department of Poultry Science Athens, GA 30602
[3.] Lincoln Premium Poultry, LLC Fremont, NE 68025
[4.] Prestage Farms Clinton, NC 28329

Ascaridia dissimilis is one of the most prevalent and economically important parasites of turkeys. With few FDA approved anthelminthics for poultry, fenbendazole is commonly used. Recently, multiple reports of decreased efficacy of fenbendazole have come to our attention. Resistance in gastrointestinal helminths has been well documented in many species and could be an emerging problem in others. This study aims to determine if fenbendazole resistance in *A. dissimilis* is an emerging problem. Worm isolates from four commercial farms were examined. For each isolate one group was treated and one group was left untreated, and groups were replicated in two separate rooms. 1-week old turkey poults were infected with 200 eggs via oral gavage. Approximately 4.5 weeks post-infection, fenbendazole (SafeGuard Aquasol, 1.25mg/kg) was administered in the water for five consecutive days to all treated groups. This dose is 1.25X the label dose and was used to ensure that all birds ingested at a minimum the full label dose. One week following treatment, birds were euthanized, necropsied, and small intestinal contents were collected for worm recovery. After quantifying worms for each individual and group, data were analyzed to determine drug efficacy within each treatment group. Three of four isolates yielded greater than 99% reduction in worm numbers as compared to the untreated controls. For the fourth, total worm reduction was only 63.9%, significantly below the expected efficacy of 99% ($p<.001$). These data demonstrate that resistance to fenbendazole in *A. dissimilis* exists within the commercial turkey industry and is potentially a growing problem.

Eimeria tenella Elongation Factor-1a (EF-1a) coadministered with chicken IL-7 (chIL-7) DNA vaccine emulsified in Montanide Gel 01 adjuvant enhanced immune response to E. acervulina infection in broiler chickens.

Alfredo Panebra

USDA

The rising demand for poultry food products is challenged by many factors including governmental restrictions on the use of antibiotic growth promoters, high density production conditions, waste management and the emergence of drug-resistant infectious pathogens; particularly those causing intestinal diseases. The use of *Eimeria spp*. oocyst vaccines has been valuable in reducing in-feed medication in chicken growth, coccidiosis vaccines based on recombinant *Eimeria spp*. genes have been shown to be effective in experimental coccidiosis. On the other hand, Montanide Gel 01, a polymeric adjuvant designed to improve safety, efficacy of aqueous DNA vaccines that trigger cellular immune response has been used to enhance DNA antigenicity. The current study aim was to explore protection rendered by *E. tenella* EF-1α and chicken IL-7 DNA vaccines emulsified in Montanide Gel 01 adjuvant to *E. acervulina* infection in broiler chickens. The criteria used to determine the effect of parasite challenge in immunized birds were like those used to evaluate drug efficacy: rate of weight gain, duodenal lesion score, oocyst shedding in chicken droppings, induction of humoral immune response and duodenal proinflammatory cytokine gene expression. Indeed, chickens immunized with EF-1α alone mostly mimicked or just lowered outcome regarding infected control group. Moreover, chickens immunized with chIL-7 alone presented a reduced oocyst shedding and increased anti-EF-1α antibody levels. Meanwhile, chickens immunized with EF-1α and chIL-7 DNA vaccine showed improvements in gain weight, reducing oocyst shedding, ameliorating lesion score and up-

regulation of pro-inflammatory cytokine gene expression. Results of this study demonstrated beneficial effects of using EF-1α DNA vaccine and/or host cytokine chIL-7 DNA emulsified with Montanide Gel 01 adjuvant improved T-cell-mediated effector function in broiler chickens challenged with *E. acervulina*.

Evaluation of a Potential Universal Subunit Coccidosis Vaccine to Protect Against Mixed *Eimeria spp* Challenge.

Maritza Tamayo[2], Emanuel Gumina[2], Ariel Sugezky[2], Sherry Layton[1,2]

[1]BV Science, 8651 Highway N, Lake St Louis, MO USA
[2]Vetanco SA, Chile 33, Buenos Aires, Argentina

Currently, there is a critical need for the development of new technologies to control *Eimeria* spp. We have developed a novel orally administered inactive subunit vaccine (BTVCx) to control *Eimeria* spp. BTVCx was evaluated in two separated mixed-*Eimeria* spp challenge trials at Southern Poultry Research. For each experiment, 1000 day of hatch chicks (Cobb 500) were randomly assigned to either the control non-treated group or the treated group (n=50/pen 10 replicate pens/group) that received BTVCx (0.2ml/bird/oral gavage) on d2 and 16 of life. On day 28 (exp1) or d21 (exp2), birds were challenged with a combination of *E. acervuline (EA), E. maxima (EM),* and *E. tenella (ET)*. Six days post challenge, 5 birds/pen birds were sacrificed, group weighed, and coccidial lesion scored according to the Johnson-Reid scale wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection. On d28 (experiment 1) or d27 (experiment 2), fresh fecal samples were collected from each pen determine the degree of oocysts shedding/cycling. Results showed significant reductions in lesions scores in both experiments 42% for experiment 1 and 45% for experiment 2 (36/39%EA, 43/39%EM, 60/66%ET, respectively) and total oocyst shedding was reduced 42% in experiment 1 and 65% in experiment 2 (40/75%EA, 68/85%EM, 40%ET, respectively). These data taken together indicate the potential of BTVCx as a control strategy coccidiosis.

Histomoniasis in broiler breeders in Peru

Nelly Cribillero, Eliana Icochea and Rosa González

Laboratory of Avian Pathology, School of Veterinary Medicine, National Major University of San Marcos.

Two outbreaks of Histomoniasis or also called Blackhead disease have been diagnosed in pullet broiler breeder flocks between 6 to 15 weeks of age. The disease is not often seen in pullet breeders in Peru, and has become important now due to no drug treatment available. The birds were submitted to the Laboratory of Avian Pathology at National Major University of San Marcos for analysis, with a background of mortality peak, diminished feed intake and depression. Necropsy findings included liver enlargement with multifocal necrosis, and typhlitis with caseous, fibrinonecrotic material or hemorrhage in lumen. Interestingly, *Eimeria tenella* were detected in ceca in both cases but presence of *H. gallinarum* were not found. In addition, treatment with nitarsone was implemented before it was prohibited, and the outbreak progressed in good terms within two weeks. Both outbreaks caused high economic losses to the farmers because of mortality in more than one flock in the farm, despite nitarsone availability. It is important to put into effect better biosecurity measures to prevent this disease.

Populations of Eimeria tenella Express Resistance to Commonly Used Anticoccidial Drugs in Southern Nigeria

Agatha Ojimelukwe

University of Port Harcort

Coccidiosis is one of the most economically important diseases of poultry. This study determined the preponderance of chicken Eimeria in southern Nigeria and assessed the parasite's resistance to three anticoccidial drugs: Amprolium hydrochloride; Amprolium hydrochloride+Sulfaquinoxaline-Sodium; and Toltrazuril. Multiplex PCR amplification of the SCAR region was used to confirm Eimeria preponderance. Resistance was assessed following the inoculation of

232000 infective oocysts into broilers. Weight gain, feed intake, feed conversion and fecal oocyst shed were recorded. At 7 days post inoculation 9 birds per treatment were sacrificed and assessed for macroscopic lesions in four intestinal regions. Percent optimum anticoccidial activity (POAA), Anticoccidial index (ACI) and Anticoccidial sensitivity test (AST) were used to access resistance. The preponderance of Eimeria spp. were E. tenella (77%), E. necatrix (55%), E. acervulina (44%) and E. mitis (11%), with multi-species infection occurring in 55% of samples assessed. Fecal oocyst shedding was low ($P < 0.05$) in the medicated groups. Lesions in the cecal region were present in all infected groups regardless of treatment and accounted for 27.8% of lesion scores by severity and 37.5% of lesion scores by frequency. Overall, lesion scores were less ($P < 0.05$) in birds of the medicated groups compared with the infected-unmedicated group. The high preponderance of E. tenella in the field, and the occurrence of cecal lesions – caused mainly by E. tenella - despite drug administration, indicate resistance in populations of this species in our isolate. Based-on the POAA, ACI and AST values, the Eimeria isolate showed reduced sensitivity to toltrazuril.

Pathology

Mycotoxicosis by T-2 toxin in broiler breeders in Peru

Eliana Icochea**,** Nelly Cribillero and Rosa González

Laboratory of Avian Pathology, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos

In three broilers breeders' flocks from 40, 45 and 65 weeks of age, from two different companies located in Lima Department, were observed necrotic oral lesions and decrease of the food consumption in apparently healthy birds. Males and females were sent to our laboratory for necropsy and diagnostic. At the clinical examination, at the beginning of the problem in the birds with 40-45 week, it was observed signs of diarrhea and necrotic oral lesions in the mouth located in the palatine area, sublingual area and loss of the tongue. At sixty week of age the birds showed feather loss and black combs. At the necropsy the most severe lesions were found in the males. Were taken samples of oral mucosa, tongue, liver, kidneys, and intestine for histopathological examination. To do the differential diagnosis with fowl pox, the samples were inoculated in egg embryos. According to the previous reports the clinical signs and gross and histopathological lesions correspond with trichothecenes mycotoxins on broiler breeders.

A Cervical Mass in an Adult Backyard Hen

Kristen Hill-Thimmesch[1], Tsang Long Lin[1], and Pat S. Wakenell[1]

[1]Purdue University Department of Comparative Pathobiology, West Lafayette, IN

A 10-year-old, buff Orpington backyard hen was humanely euthanized following a chronic history of unilateral lameness, lethargy and weight loss. Antemortem physical examination found the hen unwilling to ambulate and palpation of the breast and sternum yielded a body condition score of 1 out of 5 (1 being underweight and 5 being obese). Gross necropsy confirmed the body condition score and found few yellow, flat, 2-4mm foci on the surface of the liver, extending into the parenchyma. Most noteworthy was an ovoid mass adhered to adjacent cervical fascia, measuring 7cm x 3cm x 2.5cm and located cranial to the crop. The surface of the mass was covered in large, soft, black, tortuous vessels containing black liquid. On cut surface, the mass was soft and mottled beige to pale pink with multiple cyst-like cavities containing black to pale yellow liquid. Multifocally and randomly within the mass were firm, amorphous, pale yellow masses of material. Histopathologic and immunohistochemical stain evaluations are currently pending.

Description of round cell neoplasia in psittacine birds to characterize diagnostic features

Daniel J. Gibson[a], Nicole M. Nemeth[a,b], Hugues Beaufrère[c], Csaba Varga[d], Michael M. Garner[e], Leonardo Susta[a]

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.
[b] Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA.
[c] Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.
[d] Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada.
[e] Northwest ZooPath, Monroe, Washington, USA.

In psittacine birds (parrots, macaws, etc.), round cell neoplasms such as lymphoma, leukemia, histiocytic sarcoma are sporadic and poorly described, with only limited data addressing prevalence and specific diagnostic features. The lack of validated morphologic and immunohistochemical diagnostic criteria make accurate diagnosis of such tumors challenging, and limits further characterization. In order to characterise round cell neoplasia in psittacine birds, we retrieved archived paraffin-embedded tissues from cases of psittacine birds diagnosed with round cell neoplasia over the past 20 years. Cases were originally diagnosed at the Ontario Veterinary College, Northwest ZooPath, and the Université de Montréal. We assessed the demographic data, as well as anatomic distribution, growth patterns, cellular morphology, and immunohistochemical features of the neoplasms. A total of 41 birds with an average age of nine years, representing 14 psittacine species, were included in the analysis. Cockatiels (41%) and budgerigars (21%) were the most common species. Tumors were mainly infiltrative and multicentric, and composed of homogenous sheets of round to polygonal cells with a mitotic rate ranging from 0 to 107 per high-power field. Immunohistochemical markers for T (CD3) and B (MUM-1 and Pax-5) cells were tested on tissue microarrays derived from the 41 neoplasms, and used to determine the cell of origin. This is the first study to describe diagnostic and immunohistochemical features of a large cohort of round cell neoplasia in psittacine birds, and provides preliminary information to increase the accuracy of diagnosis and to better characterize these diseases.

Neoplasia in Small Poultry Flocks in Ontario

Elysha Smith[a], Nancy Brochu[a], Michele Guerin[b], Csaba Varga[c], Brandon Lillie[a], Marina Brash[d], Leonardo Susta[a]

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph (Ontario), N1G 2W1, Canada.
[b] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph (Ontario), N1G 2W1, Canada.
[c] Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph (Ontario), N1G 2W1, Canada.
[d] Animal Health Laboratory, Guelph (Ontario), N1G 2W1, Canada.

Small poultry flocks have become increasingly common in Ontario in recent years. Since these birds are often kept for long periods of time, and are not commonly vaccinated for Marek's disease virus, the prevalence of neoplastic diseases in these flocks might be higher than what is observed in commercial poultry. To this aim, we assessed the prevalence of neoplastic diseases in small flocks by conducting a retrospective study of postmortem cases submitted to the Animal Health Laboratory and Ontario Veterinary College from January 1998 to September 2018 (20
years). To be included, flocks had to number less than 300 birds (or be declared as a small flock in the submission form) and originate from Ontario. A total of 78 birds, ranging from 14 days to 7 years, were diagnosed with neoplastic disease, including chickens (*n* = 72), ducks (3), peafowl (1), pheasants (1), and turkeys (1). Neoplasms diagnosed included: lymphoma (30), carcinoma (28), leiomyoma (4), cholangioma (2), nephroblastoma (2), sarcoma (2), thymoma (2), astrocytoma (1) gonadal sex-cord stromal tumor (1), and peripheral nerve sheath tumour (1). Five tumours had no definitive diagnosis. Of the carcinoma cases, 14 were

adenocarcinoma of ovarian origin, while of the lymphoma cases, 21 birds had lesions consistent with Marek's disease, 5 with avian leukosis, and 4 were considered to be sporadic / nonviral. This study suggests that the most common types of neoplasia in small poultry flocks from Ontario include ovarian adenocarcinoma and Marek's disease-associated lymphoma.

Histologic and histomorphometric evaluations of disarticulation-associated femoral head separation in clinically normal broilers: Documentation of underlying predisposing cartilage abnormalities

Floyd D. Wilson[a], Phil Stayer[b], Lanny Pace[a], Frederic Hoerr[c] and Danny Magee[d]

[a]*Mississippi State Veterinary Research and Diagnostic Laboratory, CVM, MS,* [b]*Sanderson Farms, Laurel, MS,*
[c]*Veterinary Diagnostic Pathology, LLC, Fort Valley,Virginia, 22652 and*
[d]*Mississippi State Poultry Research and Diagnostic Laboratory, CVM, MSU*

Routine histologic and quantitative histomorphometric studies on femoral head separation (FHS) associated with coxofemoral joint disarticulation during necropsy were conducted on broiler chickens. The study compared groups demonstrating grossly detached femoral heads (DFH) to those with attached femoral heads (AFH). Extensive microscopic cartilage degeneration and necrosis generally compatible with osteochondrosis (OCD) was consistently appreciated along the separation surface in the DFH population. Only occasional small foci of OCD sometimes forming small clefts were observed in the AFH group. Histomorphometry disclosed significant reductions in chondrocyte density with increased pyknosis in the cartilage adjacent to the separation site and to a lesser extent in deeper regions of the growth plate for the DFH compared to AFH group. Measurement of the percent epiphyseal-physis junction occupied by either osteochondrotic defects or by separated cartilage also disclosed significant differences between groups. However, measurements of vascular canal areas within the growth plate disclosed a slight, but significant increased area for DFH compared to AFH. No differences between the groups were apparent in the occasional presence of microthrombi within the growth plate. Some results for the "FHS-disarticulation model" differ from those reported for glucorticoid-induced femoral head necrosis in broilers suggesting a possible different pathogenesis. The pathogenesis of disarticulation-FHS in broilers appears likely related to defective cartilage production resulting in increased fragility. In contrasts the proposed pathogenesis of OCD in mammals involves ischemic necrosis due to underlying vascular defects.

Production trial analysis in commercial layers using mortality survey analytics and cumulative mortality

Frederic J. Hoerr and John F. Hoerr

Veterinary Diagnostic Pathology, LLC, Fort Valley, Virginia 22652

Scheduled quarterly mortality surveys were conducted for 3 years (2016-18) in a commercial layer company with ongoing field trials. Diagnostic categories for spontaneous mortality were analyzed using a Trials feature in a poultry mortality analytics program (PathPro, https://pathpro.vetdx.com/), in which flocks were assigned to treatment or control groups. Trial A involved Gallibacterium vaccine (treatment), with the previous, same-house flock as the no-Gallibacterium vaccine control. Trial B involved F-strain Mycoplasma gallisepticum (MG) vaccine (treatment) and the previous same-house flock as the conventional MG program control. Trial C involved laryngotracheitis vaccine (treatment) with conventional program flocks on the same farm as controls. The number of birds examined were unequal between treatment and control trials. Mortality was analyzed by major diagnostic lesion, as counts of positive and negative lesion occurrence in treatment and control flocks. These data were organized by contingency table (control, treatment by diagnosis +/-) and analyzed by Chi-square test to determine whether the discrepancy between diagnostic counts was more than expected by chance ($P < 0.05$). These same flocks were analyzed for cumulative mortality by age, by pivot table

analysis of the weekly production database. Trial A vaccine was associated with lower cumulative morality with a corresponding decrease in peritonitis and salpingitis. Trial B vaccine was associated with lower mortality through peak production with a corresponding decrease in peritonitis and pneumonia-airsacculitis. Trial C vaccine was associated with lower mortality through peak production with a corresponding decrease in peritonitis. Paired analysis of production data and the diagnosis of spontaneous mortality was useful in determining outcomes of three trials involving flocks while in production.

Vaccinology

Protection Conferred by Infectious Bronchitis Virus Spike Ectodomain

Ramon Zegpi[A], Qingzhong Yu[B], Vicky van Santen[A], Haroldo Toro[A],

[A]Auburn University, Auburn, AL
[B]USDA Southeast Poultry Research Laboratory, Athens, GA

We previously demonstrated protection against infectious bronchitis virus (IBV) infection in chickens following subcutaneous vaccination with recombinant soluble trimeric recombinant spike (S)-ectodomain (e) protein. We now demonstrate proof-of-principle that vaccination with this recombinant protein delivered by a recombinant viral vector can also protect chickens against IBV infection. A recombinant LaSota strain Newcastle disease virus (NDV.Se) encoding Ark-type IBV S-ectodomain protein was generated and used to vaccinate chickens. Vaccinated chickens challenged with virulent Ark-type IBV exhibited a lower incidence of respiratory signs, a lower viral load in tears five days post challenge, and reduced tracheal damage than unvaccinated chickens.

Oral DNA vaccination with vaccine encoding turkey coronaviral spike protein containing neutralizing epitope delivered by attenuated *Salmonella* with the boost of spike protein

Tsang Long Lin, Yi-Ning Chen, and Ching Ching Wu

Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907

Turkey coronavirus (TCoV) causes atrophic enteritis and uneven growth in turkey flocks. Oral DNA vaccination with vaccine encoding TCoV spike (S) protein containing neutralizing epitopes delivered by attenuated *Salmonella typhimurium* SL1344 *aro*A mutant was carried out. *In vitro* stability of plasmid in transformed *Salmonella* after serial passages without Ampicillin was assessed by the inserted gene-specific PCR. Oral immunization of 7-day-old turkey poults with 1010 colony forming unit of transformed *Salmonella* with the plasmid coding for TCoV S476-520, S482-678, or S1-572 was followed by the second dose of transformed *Salmonella* (group 1 to 3) or intramuscular injection of 100µg purified S476-520 (group 4) or S482-678 (group 5) when turkey poults were 14 days old. After viral challenge at 28 days old, the protection efficacy was examined by enteritis-related alterations, detection of TCoV infection in ileum by immunofluorescent antibody assay (IFA), determination of S protein-specific antibody level by enzyme-linked immunosorbent assay (ELISA), and evaluation of virus neutralization (VN) titers. All plasmids remained stable in attenuated *Salmonella* after 9 passages without the selection of Ampicillin. In vaccinated turkeys, the VN titers ranged from 1:4 to 1:64 and the S-specific antibody level increased before viral challenge and no enteritis-related alterations were observed after challenge. Based on the results of IFA, group 5 showed the highest protection, 40%, followed by group 2 and 3. The results indicated that oral immunization with DNA vaccine encoding TCoV S fragment containing neutralizing epitopes delivered by attenuated *Salmonella* can provide partially protective immunity against TCoV infection.

Characterization of Mode of Action of MB-1 – a Live Hatchery Vaccine Against Gumboro Disease.

Ella Rosenzweig, Beny Perelman, Udi Ashash, Deniz Zilberman Barzilai, On Ben Tora, Ella Tiran and Virginie Loeb

Phibro Animal Health Corporation, 2 Ha'Negev st., Airport City, 6101001 Israel

MB-1 hatchery vaccine against Gumboro disease contains a naked attenuated IBD virus and is administered *in-ovo* or to day old chickens by SC injection. Previous studies suggested that following injection of the naked MB-1 virus, it is coated with maternally derived antibodies (MDA). Upon MDAs natural deterioration, the virus is released, replicates in the bursa, thus inducing the immune response. To demonstrate the MDA - dependent mode of action of the MB-1 vaccine, day-old-chicks were divided into two groups and vaccinated with MB-1: LL (low level) group containing chicks with a mean titer of 1832 and HL (high level) group containing chicks with a mean titer of 5780. The release of the virus from the immune complex and its subsequent replication in the immune organs were analyzed. In the LL group, the virus started replication in the bursa at 18 days post vaccination (PV) and reached its peak on 21 days PV while in the HL group, the virus replication in the bursa reached its peak on day 28 PV. Moreover, seroconversion in chickens from the LL group occurred 6 days earlier than in chickens from the HL group. These results demonstrate that MB-1 allows a temporal delay of the virus replication depending on MDA levels in each chicken and prove the concept of individual immunization.

Construction and Efficacy of A Recombinant HVT-ND Vaccine against NDV and MDV Challenges in SPF and NDV Challenge in Broiler Birds

Yugang Luo, Kelly Turner-Alston, Megan Bosserd, Lauren Taylor, Tyler Brown, John Dickson, Jennifer Embrey, Sing Rong*

Zoetis Vet Med R&D, 333 Portage Road, Kalamazoo, MI 49007
**Corresponding Author, sing.rong@zoetis.com*

Marek's Disease (MD) in chickens is a common cause of condemnations and immune suppression in broilers. The etiologic agent, serotype 1 Marek's disease virus (MDV) is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT), is an avirulent turkey virus that is capable of replication in chickens. HVT has been demonstrated as a useful vector for delivering major avian antigens, as well as an effective vaccine for MDV. NDV (Newcastle disease virus) causes a highly contagious and fatal disease affecting all species of birds. NDV fusion protein (F) is one of the major viral glycoproteins present in the viral envelope and is the main immuno-protective NDV antigens. We constructed thirteen HVT-ND recombinants using various promoters and poly A sequences and the target gene expression cassettes were inserted at various sites of the HVT genome. A HVT-ND recombinant vaccine was identified and selected by its excellent *in vivo* efficacy (95% protection) on Day 28 in SPF (specific pathogen-free) birds against a velogenic NDV challenge. In addition, efficacy of this vaccine against a virulent MDV challenge was observed. Furthermore, 100% NDV efficacy was observed in commercial broiler birds on Day 33. Finally, the recombinant vaccine stability was demonstrated with *in vitro* passaged viral culture by PCR, as well as IFA and DNA sequencing.

Onset of Immunity of a Recombinant HVT-ND against of a Velogenic NDV Challenge in SPF Birds

Kelly Turner-Alston, Megan Bosserd, Lauren Taylor, John Dickson, Jennifer Embrey, Sing Rong*.

Zoetis Vet Med R&D, 333 Portage Road, Kalamazoo, MI 49007
**Corresponding Author, sing.rong@zoetis.com*

A recombinant HVT-ND was developed as a bi-valent vaccine for protection against Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers. In the study with SPF leghorns, recombinant HVT-ND was inoculated in E18 eggs at target 1500 Pfu/dose. On Day 14, 16 and 19, 40 vaccinated birds of each treatment group were challenged with a velogenic NDV, respectively. Protection of 93% (37/40) was observed for Day 19 challenge. For Day 16 challenge, 85% efficacy (34/40) was observed. For Day 14 challenge, 75% efficacy (30/40) was observed. The details of experimental design and study results will be further discussed.

Evaluation of attenuated fowl adenovirus vaccines against
Inclusion Body Hepatitis in chicken

Van Dam Lai, Joo-Sung Son, Tae-sik Kim, Kang-san Kim, In-Pil Mo

Avian Disease Laboratory, College of Veterinary Medicine, Chungbuk National University, Cheongju, 361-763, South Korea

Fowl adenovirus (FAdV) is common and important infectious agent in poultry with 12 serotypes (1–7, 8a, 8b, 9–11). In Korea, Inclusion Body Hepatitis (IBH) in broiler are mainly caused by FAdV-4, FAdV-8 and FAdV-11. Strategy to control this disease is based on the maternal antibody from broiler breeder inoculated with inactivated vaccine which showed the limit duration of protection. In this study, we developed the attenuated FAdV strains from serial passages in SPF chicken embryonated egg and VERO cell including different serotype of FAdV-4, FAdV-8 and FAdV-11. The molecular changes in L1 loop of hexon and fiber genes were confirmed by sequencing. The SPF chicks were vaccinated at 3 days old and challenged at 14 days after vaccination. Birds were monitored for clinical sign and sera were collected weekly for antibody level by ELISA test. The aim of this study is to evaluate the efficacy of several attenuated FAdV strains as the vaccine in protecting broiler against IBH.

Compatibility evaluation of two Fowl Pox Vectorized Vaccines against the Avian Influenza Virus H7N3 and H5N2 administered simultaneously.

Rojo, Francisco; Castellanos, Lilia

Boehringer Ingelheim Animal Health Mexico.

In Mexico, since the 90´s decade of the last century, outbreaks of Avian Influenza virus (AIV) H5N2 were detected, and in June of 2012 highly pathogenic AIV H7N3 was identified as the responsible of the outbreak in commercial laying hens causing a severe disease with high mortality. Even the inactivated killed vaccines have been crucial for the control of the diseases produced by those viruses, the recombinant fowl pox vaccine against the avian influenza virus H5N2 has demonstrated to be a key tool in the control of this disease by stimulating the cellular response. The administration of the fowl pox recombinant vaccines against the AIV H5 and H7 is recommended at one day old, in order to demonstrate the compatibility of this two vaccines when they are administered simultaneously, in this study a challenge model efficacy study was designed evaluating the protection conferred by the vaccines against mortality and clinical signs.

A Fowlpox-vectored H7 Influenza Vaccine to Help Combat H7N3 Avian Influenza Virus Circulating in Mexico.

Mariana Sa e Silva, Justin Widener, Carlos Vega, Teshome Mebatsion, Nikki Pritchard

After a 2012 outbreak of highly pathogenic avian influenza H7N3 led to vaccination as part of the control strategies in Mexico, producers have struggled with the disease. Although vaccination generally prevents mortality, viral shedding continues, and production loses due to egg drop are common. We developed a fowlpox-vectored H7 vaccine using the TROVAC AIV H5 backbone. The vaccine protected well when administered to day old birds and can be used in prime-boost control vaccine programs as the live prime.

Seroconversion and Vaccine Take Monitoring in SPF Birds Vaccinated, Alone or Combination, with Different HVT Vector Vaccines Against Newcastle or Avian Influenza (H5 or H7) Viruses and an Inactivated Reverse Genetics Vaccine Against the Avian Influenza Virus H7N3

Luiz Sesti[1], Andrés Soto[2], Patricia Yanez[2], Samuel López[2], Fernando Lozano[3], Vilmos Palya[4]

Ceva Animal Health, [1]Latin America, [2]Mexico, 3USA, 4Hungary
luiz.sesti@ceva.com

Several groups of SPF layers (30 birds per group) were vaccinated, alone or in different vaccine combination, with three different *r*HVT vector vaccines (applied day 1, SC) expressing antigens of Newcastle virus (*r*HVT-F), avian influenza H5 virus (*r*HVT-H5), avian influenza H7 virus (*r*HVT-H7) and an inactivated reverse genetics vaccine against the avian influenza virus H7N3 (applied SC at 28 days of age). The vaccine combinations used were: a) *r*HVT-H5 + *r*HVT-H7, b) *r*HVT-H7 + *r*HVT-F, c) *r*HVT-H5 + *r*HVT-H7 + *r*HVT-F and d) *r*HVT-H7 + inactivated reverse genetics H7 vaccine. A non-vaccinated group was also used as the negative control group. All groups were maintained in isolation for the entire durations of the test. Blood samples for specific HI serology were taken weekly from 2 to 16 weeks of age from all experimental groups. Specific *q*PCR tests were carried out in spleen and feather pulp samples taken at 21 days of age for confirmation of replication of the different rHVT vectors (F/H5/H7). Data on the HI serology and *q*PCR will be presented and discussed.

Evaluation of three different vaccination programs against Newcastle disease in layers using ELISA and Hemagglutination Inhibition test

Elisa Russo[a], Sara Boldini[a], Giulia Fregnani[b], Tosi Giovanni[b], Marco Beoni[c], Laura Fiorentini[b], Marco Magrini[a], Giacomo Trevisani[c], Maria Parigi[b].

[a] MSD Animal Health Srl, via F.lli Cervi snc Centro Direzionale Milano Due - Palazzo Canova 20090 Segrate (MI) Italy.
[b]Istituto Zooprofilattico Sperimentale Della Lombardia e Dell'Emilia Romagna, Sezione Diagnostica di Forlì, Via Don E. Servadei 3E/3F, 47122 Forlì (FC) Italy.
[c]Eurovo, via Mensa 3, 48022 Santa Maria in Fabriago (RA) Italy.

Newcastle disease (ND) is a poultry pathology diffused worldwide. ND have many different clinical manifestation and highly variable pathogenicity, depending on strain virulence and host sensitivity. Many countries have governmental control plans for ND based on vaccination. Recently a recombinant turkey herpes virus (HVT) vectored ND and Infectious bursal disease (IBD) vaccine was registered in Europe. The aim of this study was to compare the serological antibody titles of three different vaccinations plans against ND using an hemoagglutination inhibition test (HI) and an ELISA test specific for the protein used in the recombinant HVT-ND-IBD vaccine. Three groups received respectively: 2 live and 2 killed vaccine (group A), 1 recombinant HVT-ND-IBD, 2 live and 1 killed vaccine (B), 1 recombinant HVT-ND-IBD, 1 live and 1 killed vaccine (C). Blood samples were collected: 20 from each group at 1, 11, 18, 25, 39, 50, 60, 70, 80, 91, 100, 107, 114, 122, 128 days. HI and ELISA tests were performed on each sample. To analyze the data a linear regression was performed on all positive samples (402) showing a moderate agreement (r=0,66). Virus neutralization test will be

executed. Using both test the trend of the 3 groups was similar. The average results of the three groups were overlapped, unless the group A that had higher titles just after the first killed vaccine, but after the second (group A) or first (groups B and C) killed vaccine they overlapped again, showing a very similar trend in all 3 groups.

Assesment of a Novel 4 Way Inactivated Vaccine for Broiler Breeders

Francisco Perozo

Merial

Newcastle disease (ND), Infectious bronchitis (IB), Infectious bursal disease (IBD) and Reovirus control requires a holistic approach that includes appropriate diagnosis, biosecurity, adequate husbandry and vaccination. Single or double administration of a tetravalent inactivated vaccine against ND, IB, IBD and Reovirus during the broiler breeder-rearing period is broadly used in Latin America. The aim of this work was to evaluate the serological response provided by a novel tetravalent inactivated vaccine (Boehringer Ingelheim) when compared with a similar vaccine under field conditions. Breeders where vaccinated by the intramuscular route at 12 weeks of age with either Boehringer Ingelheim vaccine A or other tetravalent vaccine B, serological response was tested six weeks later. The results showed consistent higher titers for vaccine A when compared to the serological response provided by vaccine B, (IBD = 9087 vs 3503), (ND = 5722 vs 1266), (IB = 3942 vs 1049), (REO= 5220 vs 509). These results indicate the suitability of using this novel vaccine as part of a control strategy for ND, IBD and Reovirus in broiler breeders.

Verification of in ovo vaccination using next generation sequencing and a traffic light- style scoring system

Elise A. Myers[a]

[a]Merck Animal Health, 2 Giralda Farms, Madison, NJ 07940

Despite widespread acceptance of in ovo vaccination and the development of a multitude of new vaccines administered by the in ovo route, in the field, questions remain about the ability to obtain adequate coverage and vaccine take. Birds were vaccinated with a dual-construct HVT vaccine and next generation sequencing was employed to evaluate vaccination using different types of in ovo vaccination equipment. First, a baseline study was conducted to compare vaccine titer levels with next generation sequencing results. Then for each group, vaccine titers were evaluated at outset, post-mixing and upon exiting vaccination equipment. Field samples were then collected to determine the overall traffic light-style score attributed to vaccinates from each group evaluated. Depending on the score achieved, any remedial actions required at hatchery level are provided in a color-coded report. The ideal timing for feather pulp sampling post vaccination was previously determined by generating a standard curve. This traffic light-style approach provides a convenient way to evaluate vaccination preparation, efficacy of equipment and assessment of overall flock coverage for hatchery veterinarians.

Optimization of antibody avidity index estimation using logistical regression models

Ramon Zegpi, Ash Abebe, Stephen Gulley, Vicky van Santen, Haroldo Toro.

Auburn University. Auburn, Alabama.

The quality of immunization can be partly characterized by the avidity of antibodies produced after vaccination. Avidity is the sum of strength of all affinities between antibodies and antigens. It is generally estimated using a modified ELISA, by adding a chaotropic agent in a gradient of

concentrations to inhibit the antibody-antigen interaction. The concentration of the chaotropic agent to reduce the interaction halfway between maximum and minimum value is called avidity index. Avidity index is an estimator of avidity. After vaccination, a high avidity is desirable. In previous work in our lab, a polynomial regression was used to estimate the avidity index of serum antibodies obtained from chickens vaccinated against Infectious Bronchitis Virus (IBV). Polynomial regression does not favor reproducibility, since little variations in ELISA results can produce dramatic changes in the shape of a polynomial and, therefore, in avidity index values. To improve our analyses, we tested non-linear logistical regressions and 2 different chaotropic agents to estimate avidity of chicken IBV-specific antibodies. Logistical regressions are less sensitive to little variations of ELISA values. Potassium thiocyanate (KSCN) and Guanidine Hydrochloride (GuHCl) were tested as chaotropic agents. Our results show that a 5 parameter logistical regression is the best model for the non-linear regression and GuHCl provides more reliable results. A software was generated to standardize the analyses of results, it can be obtained for free (https://github.com/rzergpi/Avidity).

Development of a Genotype-Matched Vaccine Against Virulent Newcastle Disease Virus

Dana Goldenberg, Qingzhong Yu, David L. Suarez, Claudio L. Afonso*

Exotic and Emerging Avian Viral Disease Research Unit, Southeast Poultry Research Laboratory, US National Poultry Research Center, ARS, USDA, 934 College Station Road, Athens, GA 30605, USA

Newcastle disease virus (NDV) has two surface glycoproteins that induce neutralizing antibodies: the hemagglutinin-neuraminidase (HN) and the fusion (F). All NDVs are members of one serotype, yet, despite heavy vaccination, NDV causes devastating disease in poultry worldwide. Several studies have concluded that homologous (genotype matched) vaccines, have been demonstrated to have advantages in control of viral shedding and survival after challenge of sub-optimally vaccinated chickens. Virulent field strains isolated in the Middle East belonging mainly to class II genotype VII, sub-genotypes VIId predominant during (2007 to 2010), VIIi during (2011 to 2013) and more recently, sub-genotypes VIIj is becoming the dominant genotypes (2011 to 2018). All genotype VII viruses are predicted to be virulent. Here we describe the utilization of a reverse-genetics technique to develop a recombinant NDV that expresses the HN and F genes of genotype VIIj virus, using the LaSota vaccine strain as a backbone. We amplified and cloned the genomic region comprising the HN and F genes of the sub-genotype VIIj. In addition, we altered the virulent cleavage site of the VIIj F gene to match the sequence of the low-virulent vaccine strain LaSota, and rescued the recombinant virus. Evaluation of the immunogenic efficiency and protective capacity of the new recombinant virus in vaccination-challenge animal experiments will discussed.

Virology

IBV DMV/1639/11 causing cystic oviducts in egg layers: Prevalence and risk factors in Eastern Canada laying flocks

Eric Parenta and Martine Boulianne[a]

[a]*Chair in Poultry Research, Faculté de médecine vétérinaire, Université de Montréal.*

IBV genotype DMV/1639/11 emerged recently in the Eastern Canada, instigating significant losses to the egg industry by causing cystic oviducts and the ‘‘false-layer syndrome’’. The objectives were to determine the prevalence of this genotype in the area and risk factors associated with positive sites. The prevalence was determined by sampling 34% of the sites (52/153 sites) in the province of Quebec, Canada by using a proportionate stratified random sampling design. Risk factors were evaluated by univariable logistic regressions with the site status (positive or negative) as the outcome and various site characteristics as factors. The prevalence was estimated at 18.9% (C.I. 95% 9.9-27.8%) with positive sites identified in 3/12 counties of the province. The flock’s age was significantly

associated (p=0.02) with the outcome (OR 0.92 [0.86-0.98]), where an older flock had lower odds for being positive for DMV/1639/11. A trend (p=0.1) was observed between the DMV/1639/11 status and the presence of broiler chicken houses on site (OR 3.42 [0.74-15.03]). No associations were observed (p>0.05) between the DMV/1639/11 status and the size of the flock (OR 1.00 [1.00-1.00]), the number of houses on site (OR 0.92 [0.40-1.69]) or the presence of replacement laying pullets on site (OR 0.81 [0.22-2.94]). In conclusion, the localization of positive sites and regions will allow the industry to implement control measures to limit the propagation of this genotype in the area. Risk assessment of biosecurity and vaccination protocols will be necessary to further identify factors associated with positive sites for the DMV/1639/11 genotype.

Virus neutralization of the Canada/17-099269/17 strain of Infectious Bronchitis Virus using antisera to known vaccine strains

Andrew Bishop

University of Georgia PDRC

In Canada, several IBVs have been detected circulating in broiler and layer flocks across the breadth of poultry producing regions, with a California/1737 like virus consistently the second most prevalent virus detected over the last decade. For this reason, our laboratory obtained a clinical sample from a diagnostic laboratory in Canada that had been molecularly typed as a Cal/1737 variant. Our laboratory isolated a virus from this sample, named Canada/17-099269/17, and performed full S1 sequencing which determined that this virus was only 88% similar to the Cal/1737 strain, causing us to question whether the current vaccination strategy in Canada could protect against this virus. To begin the evaluation, we explored whether significant neutralization titers of Canada/17-099269/17 might be achieved using antisera to Massachusetts and 793B group vaccine strains, as well as antisera to the Cal/1737 virus, using a beta-method neutralization in chicken embryos. The Canada/17-099269/17 virus was mixed with serially diluted antisera against the two vaccine strains, the Cal/1737 strain, and a 50/50 mixture of antisera against the Mass and 793/B vaccine strains. Each virus/antisera dilution was inoculated into 9-11-day-old SPF chicken embryos via the chorioallantoic sac route, and checked for lesions and mortality at 7 days post-inoculation. Results of these neutralizations will begin to demonstrate if antibodies against these vaccine strains improve neutralization titers, and whether a simultaneous combination of both vaccine strains might create an additive effect.

Broiler Study Evaluating Protection Against A Contemporary DMV/1639 Isolate By A Commercial GA08 Vaccine Either By Itself Or In Combination With Other IB Vaccines

Kalen Cookson[1], Manuel Da Costa[1], John Dickson[1] and Jon Schaeffer[1]

[1]Zoetis US Poultry, Durham, NC

A previous study in SPF leghorns demonstrated this GA08 vaccine gave excellent cross protection against a reference DMV/1639 challenge isolate. This study was conducted to compare IBV protection in broilers vaccinated with GA08 vaccine by itself or in combination with other serotypes against contemporary 1639 isolate DMV/240/18. 450 day of age broilers were divided into 5 groups of 5 isolators each and vaccinated with live B1 and the following IB vaccine(s): GA08, GA08+GA98, GA08+Ark, GA08+Mass and Mass. Choanal swabs were taken weekly from vaccinated controls. At 25 days of age, 3.5 EID50 DMV/1639 IBV challenge was given via eye/nose drop. At 30 days of age, all birds were bled, evaluated for clinical signs and scored for internal lesions; tracheas were swabbed for IBV PCR and preserved in formalin for histopathology. GA08 or Mass gave the lowest IBV seroconversion but GA+Mass gave the highest. All birds were strongly positive for vaccine “take” at 7 days. Clinical signs were very mild and there were no significant differences. Mass by itself gave the lowest protection levels (71%) against significant IBV loads (Ct≤30) while all the GA08 combos were significantly higher (90-100%) and GA08 by itself was intermediate (81%). Using the “VI negative” Ct≥35 cut-off, GA08 protection went down but

GA08+GA98 and GA08+Mass both remained significantly higher than Mass (33%) with protection levels of 76% and 81%, respectively. While Ark vaccine combined with GA08 did enhance protection against infection, it came at a cost of more tracheal lesions and vaccine persistence at 30 days.

Genetic and Pathologic Characterization of Recombinant TC07-2-like and Q1-like Infectious Bronchitis Viruses in Korea

So Youn Youn, Il Jang, Hyun Jeong Lee, You Chan Bae, Seong Joon, Joh, Hyun A Kim, Jong Sun Jang, Yong Kuk Kwon, Ji Youn Lee

Avian Disease Research division, Animal and Plant Quarantine Agency , 177 Hyeokshin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, Republic of Korea

Infectious bronchitis virus (IBV) is a highly contagious respiratory pathogen of chicken and causes significant economic losses due to poor production performance in poultry industry around the world. Due to its capacity of spontaneous mutation and genetic recombination, new variant strains have emerged continuously. Among various IBV variants, TC07-2 and Q1 IBV have been known by enteric strains related proventriculitis, and reported in different Asian and Europe countries including Korea. In this study, we performed genetic and pathologic characterization of TC07-2 and Q1 isolates, designated KrD1515 and KrD0564, respectively. Phylogenetic analysis revealed that theses two isolates are genetic recombinant strains harboring N gene from non-TC07-2 and non-Q1 genotypes. In animal experiment, clinical signs and pathologic lesions have been observed restrictively in respiratory systems. In addition, virus shed longer through oral route than cloacal secretions. Our results suggest that the evolution of IBVs by genetic recombination may affect altering of tissue tropism and pathogenecity in chickens.

Intestinal tropism of an IBV isolate is not explained by spike protein binding Specificity

Farjana Saiada[a], Vicky L. van Santen[a], H.L. Shivaprasad[b], Charles Corsiglia[c], Rodrigo A. Gallardo[d]

[a] Department of Pathobiology, 264 Greene Hall, College of Veterinary Medicine, Aubur University, Auburn, AL 36849-5519 USA.
[b] California Animal Health and Food Safety Laboratory Tulare Branch,
[c] Foster Farms Livingston, California.
[d] Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis.

An infectious bronchitis virus (CalEnt) with unusual tissue tropism was isolated from a California broiler flock exhibiting runting-stunting syndrome. IBV was detected in the small intestine, but not in respiratory tract or kidney. During virus isolation in embryos, it did not replicate in CAM, but could be recovered from intestines. Its S1 protein had 94% aa sequence identity to Cal99. Intestinal lesions were reproduced following ocular/nasal inoculation of SPF chickens, but respiratory signs and lesions were also present. Virus was detected in both respiratory and intestinal tissues. In the present work, to determine whether the novel tropism of IBV CalEnt was due to increased ability of its S1 protein to bind to intestinal epithelium, we compared binding of soluble trimeric recombinant S1 proteins derived from CalEnt, Cal99, and ArkDPI viruses to fixed tissues. Contrary to expectations, CalEnt S1 protein did not bind to small intestine. Unlike Cal99 and ArkDPI S1 proteins, it also did not bind to respiratory epithelium or CAM. Using only the CalEnt S1-N-terminal domain or including the S2- ectodomain (lacking membrane and cytoplasmic domains), which both improve binding of ArkDPI S1, did not lead to detectable binding to any tissue tested. Our results indicate no/poor binding of the CalEnt spike protein to both respiratory and intestinal tissues and thus do not support better attachment to intestinal epithelial cells as a reason for CalEnt's extended tropism. Our negative results might reflect shortcomings of the assay, including that it does not detect potential contributions of the S1-C-terminal domain to attachment.

SEROLOGY AND MOLECULAR DIAGNOSTICS AS TOOLS TO ASSESS LOSSES INDUCED BY INFECTIOUS BRONCHITIS (IB) IN BROILER FARMS

Jorge Chacón[1], Leandro Bianchet[1], Graziela Santos[1], André Miyazaki[1], Luiz Sesti[2]

Ceva Animal Health - [1]Brazil, [2]Latin America

The BR-I variant strain of Infectious Bronchitis Virus (IBV) has caused significant production losses to the Brazilian poultry industry because the low genetic homology with the strain used for vaccination: Massachusetts. In order to measure production losses caused by BR-I strain in broiler flocks previously immunized with Massachusetts vaccine, IB ELISA serology (Idexx; blood samples taken at 6 weeks of age) and RT-PCR assay specific for the BR-I strain were used to distinguish between infected and non-infected flocks in eight companies. The RT-PCR assay showed to be the most accurate method to detect infected flocks. However, two companies presented high molecular detection of the BR-I strain (93.75 and 96.3% of positivity) making it difficult to measure the economic impact in those farms. Then, the evaluated flocks of these two companies were divided in two groups according to their ELISA Titers: 24 flocks (GMT _1500) and 19 flocks (GMT <1500). These flocks were labeled early and late infected flocks, respectively. The 1500 GMT was used as a cut-off titer based on previous serology surveys. Flocks presenting GMT _1500 (early infections) presented lower productive (lower daily weight gain and viability, and higher feed conversion and use of antibiotic) and slaughterhouse (condemnations of airsacculitis and colibacillosis) parameters than late infected flocks (GMT <1500). Collectively, these data show that IB ELISA titers at the end of the broiler cycle and RT-PCR of variant IBV correlate well with losses in production and processing parameters in broilers not appropriately protected against the Brazilian BR-I strain.

CONTROL OF PRODUCTION LOSSES IN COMMERCIAL LAYER FLOCKS INFECTED WITH THE BR-I VARIANT STRAIN OF INFECTIOUS BRONCHITIS VIRUS IN BRAZIL

Jorge Chacón 1, Fernando Resende 1, Haiala Coelho 1, Leandro Bianchet 1, Priscila Rocha 1, Luiz Sesti 2

Ceva Animal Health – 1 Brazil, 2 Latin America

The Brazilian BR-I variant strain of Infectious Bronchitis Virus (IBV) is the most prevalent and geographically distributed in Brazil. Vaccination programs using Massachusetts vaccine strains will not control the BR-I strain since these strains present a low genetic and antigenic homology. Recently, a live attenuated IB vaccine containing the BR-I strain was approved for use in Brazil. For evaluation and comparison of IB immunization programs, against the BR-I strain, based on homologous (BR-I) and heterologous (Mass) vaccines, eight-layer farms were monitored. During the rearing period, the flocks vaccinated with the BR-I strain had evident lower clinical respiratory disease, mortality rate and antibiotic use. In addition, they presented higher body weight and weight uniformity at the end of the rearing period. In the production period, the flocks immunized with BR-I showed a clearly better general health status expressed by an early beginning of egg production which was at least three weeks before the flocks vaccinated with the Mass strain only. External and internal egg quality of eggs produced by layers vaccinated with the BR-I strain vaccine was evidently improved. Homologous BR-I strain vaccinated flocks produced at least 3% less eggs with deformed, dirty and cracked shells and also presented a better uniformity of egg weight than those vaccinated with Mass vaccine only. The results obtained from these field evaluations confirmed, and are according to, the scientific reporting that homologous IB vaccination (vaccine strain genetically and antigenically similar to the field strain) is a critical point for effective control of field IBV.

Infectious Bronchitis Virus (IBV) in Peru: a 10-year molecular epidemiological survey (2009 to 2018)

Claudia Carranza[1], Luis Alzamora[1], Juan Carlos[1], Antonio J. Piantino[2], Sjaak de Wit[3], Luiz Sesti[1].

[1]Ceva Animal Health, Peru.
[2]Avian Pathology Laboratory, University of Sao Paulo, Brazil.
[3]GD Animal Health, Netherlands. 4Ceva Animal Health, Latin America

Infectious Bronchitis disease, and its etiological cause (different IBV strains), has been present in Peru for a long time. Since the first IBV strain was detected in 1968, substantial production losses have been a constant burden to the Peruvian poultry industry. A quite reliable control of IB can be achieved with good biosecurity measures, adequate management and efficient vaccination programs. In recent years, since conventional vaccination programs with conventional vaccines containing Massachusetts strains have failed to induce proper immune protection, the presence of circulating variant IBV strains was suspected. The present epidemiological report analyzes molecular diagnostic data (RT-PCR and either sequencing or specific RFLP protocols) obtained during a period of 10 years of IBV monitoring in Peru. A total of 195 samples were taken from flocks housed in 6 different states (northern, southern and mid part of the country) comprising 14 zones of poultry production. Samples were collected from 3 types of industrial poultry (broilers, breeders and commercial layers) presenting an age spread of 2 to 78 weeks. The results show a quite high detection rate for IBV variants (67.8%) directly correlated to clinical disease, particularly, respiratory disease in broilers. A clear tendency is observed regarding the diagnostic frequency of variant IBVs throughout the period of investigation. The Chinese Q1/J1-like variant strain is the predominant IBV strain representing almost 35% of all detections. On the other hand, although the 793/B-like strain represents only 9.2% of the total number of detections during the past 10 years, it was the predominant IBV strain detected during 2018 (41.9%) and was found only in long-living birds (layers and breeders). These results are the first report of the detection of a 793/B-like IBV variant in Peru. These strains are suspected to be from vaccine origin. Bayesian analysis for evolutionary divergence is underway to determine whether genetic relationship with other 793/B-like strains detected in the continent exists. Finally, the Brazilian BR-I variant strain has also been detected in 2017, confirming the large distribution of this strain in South America. Details from this epidemiological survey will be presented and discussed along with the economic implications for the Peruvian poultry industry.

Economic estimation of direct disease cost of an IBV outbreak in a grandparent flock in Brazil

Claudia Carranza[1,2], Claudete S. Astolfi-Ferreira[2], Antonio J. Piantino[2]

[1]Ceva Animal Health, Peru.
[2]Avian Pathology Laboratory, University of Sao Paulo, Brazil.

Infectious Bronchitis virus is present in poultry farms worldwide and while vaccination is a common practice, the emergence of new variants that overcome the birds' immunity due to the high immunological pressure is frequent and often results in outbreaks of the disease. The effects of an outbreak of IBV can affect the respiratory, renal and reproductive systems of broilers, layers and breeders, leading to severe economic losses to the industry therefore placing Infectious Bronchitis as one of the most important diseases in poultry. However, published data concerning the economic estimation of these outbreaks is scarce and the real quantification of the effects is often unknown. Our results show the direct disease cost of an outbreak of IBV in a broiler breeder grandparent unit in Brazil during the last semester of 2013. This outbreak of IBV affected 62.5% of the nucleuses at a grandparent facility of 178 883 birds. For the analysis of the direct disease cost we considered the valuation of the effect of IBV on zootechnical parameters such as egg production, incubable eggs and hatchability during a period of 5 weeks. Birds between 26 and 49 weeks of age were affected. Observed production drop ranged between 5 – 10%, while the hatchability drop was on average 22.75%

(with flocks presenting drops of more than 50%). Embryo late mortality was also altered as well as allocated feed consumption time.

Persistence of Infectious Bronchitis and Newcastle Disease Vaccine Virus by In Situ Hybridization.

Banda, A[a]., E. Orozco[a], R. Mackey[a], and I. Alvarado[b], Y. Zhang[a], and L. Yan[a].

[a]Poultry Research and Diagnostic Laboratory., College of Veterinary Medicine. Pearl, Mississippi 39288. bMerck Animal Health Watkinsville, GA 30677.

The goal of this study was the evaluation of the vaccine reactions induced by infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) vaccines applied as a single application or in combination. Two hundred and forty one-day old commercial broilers were assigned into 12 groups with two replicates. Commercially available IBV live vaccines Ma5, M41, and NDV vaccines C2, B1, and VG/GA were included. Vaccines were applied as single or combined applications. At seven and fourteen days of age birds were humanely euthanatized and portions of trachea were fixed in buffered formalin and paraffin embedded. Two antisense digoxigenin-labeled riboprobes targeting the matrix gene of NDV, and the region 5'-UTR of the IBV to perform in situ hybridization (ISH) techniques on formalin-fixed, paraffin-embedded tracheal tissues. The presence of the virus detected by ISH was associated with histological changes in the trachea. Differences in the persistence of IBV and NDV were detected by ISH. The viral persistence was higher at seven days than at 14 days postvaccination. Differences among the different treatments are discussed.

Seroconversion Response of Commercial Broilers Vaccinated with Two Vaccination Programs against Newcastle Disease

Bruno Garcia [1]

[1] Veterinary School, University of San Marcos

In order to estimate vaccination efficacy against Newcastle disease (ND), the seroconversion response for ND of commercial broilers vaccinated with two vaccination programs at two different periods was compared in a poultry company in Peru. In Period 1 (January 2011 - June 2012; 18 months) a total of 61.7 million broilers were vaccinated at day 1 with an inactivated oily vaccine (subcutaneously) and a conventional live vaccine (HB1 strain; spray) plus two revaccinations in the field with a conventional live vaccine (La Sota strain; drinking water) at 10 and 20 days of age. In Period 2 (July 2012 - December 2017; 66 months) a total of 338 million broilers were vaccinated in the hatchery only with a vector *r*HVT-F vaccine (70% *in ovo* and 30% subcutaneously) plus a conventional live vaccine (HB1 strain; spray). Blood samples for ND Elisa serology (Idexx kit) were collected in the field at slaughter age for both periods. A total 4,554 and 19,890 serum samples were analyzed from Period 1 and Period 2, respectively. The Geometric Mean Titer for Period 1 (2744 for 253 flocks) was higher ($p<0.05$) than for Period 2 (1799 for 1142 flocks). A quite evident downward and uniform effect on seroconversion was observed in Period 2. These results indicate that the vaccination program used in Period 2 induced a more predictable efficacy against ND.

Virulent Newcastle disease virus efficiently replicates in chicken feathers and is readily identified by rRT-PCR and virus isolation

Kiril Dimitrov

USDA/ARS/USNPRC/SEPRL

The presentation will focus on the use of feathers as clinical samples to identify Newcastle disease virus during surveillance and disease outbreaks. NDV efficiently replicates in feather epithelium and also

remains viable in feathers of dead birds for several weeks. NDV is identified in high titers in feather of infected birds. This work will also elaborate on impact of feather collection time point on viral loads and also the affect of vaccination of virus spread and replication in feathers.

Evaluation of factors influencing Marek's disease vaccine titer variability

Blanca López de Juan Abad, Aneg L. Cortes, Isabel Gimeno

College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Marek's disease (MD) vaccines are cell-associated and therefore require special care while handling, storing, and administering. The goal of this study was to asses dose uniformity in commercial MD vaccines and to evaluate the effect of three factors of vaccine production and reconstitution which contribute to increased dose variability: time, poor homogenization of vaccines, and infectivity rate. In a study evaluating 42 vaccines, the coefficient of variability (CV) of vaccine dose ranged from 10 to 59% and was greatly affected by inappropriate mixing of the vaccines. In addition, since not all vaccines were equally affected by poor mixing, the effect of infectivity rate (IR) was studied. IR is defined as the percentage of cells infected with viable vaccine virus in a vaccine vial and calculated as the average PFU per number of live cells at time of titration (avg PFU/ # alive cells). To assess the effect of time and homogenization, vaccines were titrated immediately after reconstitution, and 1 hour post reconstitution by titrating them under two conditions: continuously mixing with magnetic stirring and not mixing. To assess the effect of IR on dose variability, two approaches were used. First, commercial vaccines were titrated and CV was calculated before and after adding non-infected chicken embryo fibroblasts to decrease IR. Secondly, various dilutions of a commercial vaccine were grown in CEF to obtain various IR and then titrated to calculate CV. We demonstrate an inherent variability in commercial vaccines titers and the correlation between each factor and CV is discussed.

Vaccinal efficacy of Gallid alphaherpesvirus 3 strain 301B/1 from BAC clone against Marek's disease virus challenge

Taejoong Kim[1], John Dunn[2], Stephen Spatz[1]

[1]U.S. National Poultry Research Center, USDA-Agricultural Research Service, Athens, GA 30605
[2]U.S. National Poultry Research Center, USDA-Agricultural Research Service, East Lansing, MI 48823

Marek's disease is a highly contagious lymphoproliferative disease in chickens. Marek's disease virus (MDV) has evolved its virulence partly because of imperfect vaccines and faulty administraion. Turkey herpesvirus (HVT) and Gallid alphaherpesvirus 3 (GaHV-3) have been developed as bivalent vaccines to improve the level of protection elicited by single formulations of HVT or GaHV-3 (e.g. SB-1 vaccine strain). Since in vitro passage of MD vaccine strains can result in over attenuation, we sought to secure a molecularly defined MDV vaccine strain by inserting the mini-F replicon into the genome of another GaHV-3 strain (301B/1) creating a bacterial artificial chromosome (BAC). Infectious virus was rescued from various 301B/1-BAC clones by reverse genetics techniques. Reconstituted 301B/1-BAC viruses showed growth kinetics comparable to parental 301B/1 virus. Preliminary data from a vaccine protection study using specific pathogen free chickens suggest vaccine virus reconstituted from a selected GaHV-3 301B/1 BAC exhibited an efficacious protection profile against very virulent MDV challenge. Further details of protective efficacy of the molecularly cloned vaccine candidate will be discussed.

Use of HVT vector to express protective antigens from important viral pathogens

Sanjay M. Reddy, Owais Khan, Mohammad Al-Mahmood and Blanca Lupiani

Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843

The use of turkey herpesvirus (HVT) based vector vaccines for protection against economically important diseases in chicken is widely accepted. In the present report we describe the development of two HVT vectors expressing the following protective antigens: fusion (F) gene of Newcastle disease virus (NDV) or spike (S) gene of infectious bronchitis virus (IBV). Both recombinant HVT vector vaccines were stable after *in vitro* propagation in chicken embryo fibroblasts. In addition, in order to develop vector vaccines capable of simultaneously protection against important respiratory and immunosuppressive diseases of poultry, we identified two novel sites in the HVT genome where foreign genes could be cloned, and identified regulatory gene sequences that allowed the expression of high levels of two foreign genes in a single HVT vector, without affecting HVT replication. The F gene of NDV was therefore cloned in a previously developed HVT-VP2 vector. The expression of F simultaneously with VP2 was very high. The *in vitro* results obtained until now show that it is feasible to use HVT as a vector to express multiple protecting antigens for several immunosuppressive and respiratory poultry viruses. Our preliminary work showed that the HVT-VP2 vector produced protective antibody levels in vaccinated chickens. It is our expectation that, the new vectors described here will be able to protect vaccinated chickens against important respiratory and immunosuppressive diseases.

Current infectious bursal disease virus circulation in Mexican poultry. A molecular survey

Rios-Cambre, JF; Cabriales-Jimenez, JJ; Trejo-Martínez, EM; Medina-Jaime, FA; Romero-Domínguez, G.

An extensive survey was conducted in the most important broiler producing regions in Mexico. Bursal samples were taken in FTA cards and shipped to the lab for PCR detection and sequencing. The aim of this study was to update the knowledge on which strains are most prevalent and to which cluster they belonged. It was proven that most IBD virus strains belonged to the cluster that was formerly known as US variants. This information might be useful for designing effective vaccination procedures for control.

New Introduction of a Genome-Reassortant, Very Virulent Infectious Bursal Disease Virus in the USA.

Daral J. Jackwooda,[b*], Linda O. Michel[a], and Michelle L. Kimber[c]

[a]Food Animal Health Research Program, [b]Department of Veterinary Preventive Medicine,The Ohio State University/OARDC, Wooster, OH 44691. [c]Larad, Inc., BioHio Research Park, Wooster, OH 44691, USA.

In 2018, bursa tissue samples from a pullet flock in New York State that was experiencing immune suppression related disease were sent to our laboratory. The virus identified was a reassortant very virulent infectious bursal disease virus (vvIBDV) which had a vvIBDV genome segment A and a non-vvIBDV genome segment B. The first identification of vvIBDV in the United States was in California in December 2008. Since that time, several outbreaks of both true vvIBDV and genome reasserted viruses have occurred in broilers, pullets and backyard chickens in northern and southern California. These viruses did not spread outside California until 2014, when a reassorted vvIBDV was identified in a pullet flock located in Washington State. Based on their genome segment A nucleotide sequences, the California and Washington State viruses were

related to the type strain of vvIBDV (UK661) and to each other. The genome segment A sequence of the 2018 New York vvIBDV is not related to the California and Washington State vvIBDV indicating it is a new introduction into the U.S. A phylogenetic analysis indicated this virus was most similar to vvIBDVs from Morocco (GenBank #AVZ47149), Iran (GenBank #ANT46036) and China (Genbank #AAN04903). The New York vvIBDV caused 100% morbidity and 68.7% mortality when it was used to challenge 4 week old specific-pathogen-free (SPF) layer chicks. Gross lesions in the bursa of the SPF birds consisted of large edematous bursas that were yellowish in color and some had hemorrhages on the serosal and mucosal surfaces. Microscopic lesions included inflammation, severe lymphocyte necrosis, atrophy of the follicles and follicular depletion of lymphocytes.

Towards the Construction of Sigma C epitope-based vaccine for avian reovirus

Joseph.J. Giambrone[a] Hongzhuan Wu[b], Lula Smith[b], Karyn Scissum Gunn[b], Robert Villafane[c] , B K Robertson[c],

New antigenic reoviruses (ARVs) continue to emerge resulting in vaccine failures. The ARV σ C protein is the main immunogenic surface protein of ARV. Epitope-based vaccine prototypes can stimulate protective immune responses. A protocol for epitope prediction for an ARV was developed based upon: 2D and 3D structural analysis, PROSITE glycosylation patterns, protein sequence homology and alignments, and hydrophobic index. Bioinformatics analyses of ARV σC protein revealed putative epitopes at 3 locations. The neutralization activity of these 3 expressed σC deletion fragments needs to be determined before the construction of a Sigma C epitope-based vaccine against ARVs can be accomplished.

Pathogenicity of a variant Reovirus isolate close to Classical strains obtained from Clinical case of Viral Arthritis in Northwest Canada.

Victor A. Palomino-Tapia[a], Catalina Barboza-Solis[a,d], Darko Mitevski[b], Tom Inglis[c], Mohamed F. Abdul-Careem[a],*

[a]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N4N1, Canada
[b]Poultry Health Services, Airdrie, AB T4A2G8, Canada
[c]The Institute of Applied Poultry Technologies, Airdrie, AB T4A2G1, Canada
[d]National University of Costa Rica, Heredia, Costa Rica.

In the last 6 years, avian reovirus (ARV) induced Viral Arthritis (VA) has emerged as an economically important disease in broiler flocks in Western Canada. Control of the disease is achieved by the vaccination of parent stocks with ARV autogenous vaccines containing isolates from the most important variant clusters. However, ARVs from the same cluster as classical vaccines but from a different sub-cluster (Sub-cluster 1.2) have been thought to be somewhat protected by classical vaccines and therefore not included in autogenous vaccine programs. To evaluate the pathogenicity of cluster 1.2 ARV, the clinical isolate 17-0160 was selected. At the day of hatch, broilers obtained from breeders vaccinated with a commercial ARV program were challenged by the footpad route at two different titers: 104.0, and 105.0 TCID50 per bird. The birds were placed on floor pens and monitored daily for clinical signs (CS). Cloacal swabs were obtained at 7 and 14 days post inoculation (DPI). Weights and footpad measurements were collected daily. At 14 DPI, all birds were euthanized. Tendon, footpad, heart, and intestine samples were collected for histopathology. Tendon, footpad, spleen, and liver were collected for qPCR. Upon comparison with the control group, the 104.0 TCID50 group had 33% lower weight ($p<0.05$), 56.9% increase in Footpad:bodyweight ratio ($p<0.05$), and an average CS of 1.08 ($p<0.05$). The 105.0 TCID50 had 27% lower weight ($p<0.05$), 46.4% increase in Footpad:bodyweight ratio ($p<0.05$), and an average

CS of 1.83 ($p<0.05$). Histology and qPCR data will be discussed at the presentation.

Characterizing the etiological agent of guinea fowl viral pancreatitis

Jean-Luc GUERIN[a], Léni CORRAND[b], Guillaume CROVILLE[a], Marie-Noëlle LUCAS[a], Luc ROBERTET[a], Marie SOUVESTRE[a], Guillaume LE LOC'H[a], Céline LOPEZ-ROQUES[c], Vincent BLONDEL[d], Maxence DELVERDIER[a]

[a]ENVT, INRA, ENVT 23, chemin des Capelles, Toulouse, France
[b]Anibio, ENVT, ENVT 23, chemin des Capelles, Toulouse, France
[c]Plateforme Génomique, INRA, Castanet Tolosan, France
[d]SOCSA Elevage 32, Mirande, France

Viral pancreatitis of guinea fowl is a pathological condition reported for several decades in France and in the world. Although the disease and its pathology is well described, its viral etiology remains poorly characterized. Previous works had shown that it is an avian adenovirus. On the basis of several clinical cases of pancreatitis on Guinea fowl in southwestern France in 2017, the fine genetic characterization of the virus was undertaken. Several clinical cases were included and clinical and pathological data were recorded. Histopathological analysis confirmed the typical lesion pattern, including intranuclear inclusions. We performed complete genome sequencing directly from pancreatic pathological tissue using a high throughput sequencing approach. We generated 764,112 reads, of which 4,781 (0.63%) corresponded to viral sequences, and assembled a complete genome of 41,729 bp. Phylogenetic analysis showed that this virus belongs to Fowl adenovirus type 1 (FAdV-1) and is very closely related to chicken apathogenic adenovirus (CELO) and gizzard erosion syndrome viruses. Sequencing of the gene encoding the hexon protein was applied to all cases included in the study to provide additional insight into the genetic diversity of FAdV-1 associated with guinea fowl pancreatitis. Genetic characterization of guinea fowl pancreatitis adenovirus suggests that this virus is probably shared with other galliform species. Broader investigations will better document the dynamics of infection in guinea fowl and with other poultry species and thus propose appropriate control measures.

Defining the Host Range of Aquatic Bird Bornavirus through *In Vitro* Replication Kinetics and Lesion Development *In Ovo*

Alexander Leacy[a], John Pham[a], Li Deng[b], Eva Nagy[a], Csaba Varga[c], Nicole M. Nemeth[d], Leonardo Susta[a]

[a]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph (Ontario), N1G 2W1, Canada.
[b]Department of Pathology and Molecular Medicine, Faculty of Health Science, McMaster University, Hamilton (Ontario), L8N3Z5, Canada.
[c]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph (Ontario), N1G 2W1, Canada.
[d]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, (Georgia), 306059, USA

Aquatic bird bornavirus (ABBV) causes ganglioneuritis and encephalitis in waterfowl species, and has been demonstrated to be highly prevalent among migratory waterfowl in North America. ABBV strains have been sporadically isolated from other birds, including gulls, bald eagles, and emus, suggesting a broad host range. Given the ability to infect multiple species and widespread distribution through migratory birds, strains of ABBV may have the potential to infect commercial poultry (i.e., chickens and turkeys). In this study, we aimed to evaluate the host restriction of ABBV for poultry species. First, we conducted single- and multi-step growth curves to assess the ability of ABBV to grow in primary chicken, turkey, duck and goose embryo fibroblasts (CEF, TEF, DEF, GEF, respectively), as well as immortalized DF-1 (chicken), CCL-41 (duck) and QT35 (quail) cell lines. Second, host restriction was assessed *in ovo*. Groups of duck, chicken and turkey eggs (n=20) were inoculated with ABBV into the allantoic cavity and yolk sac, and embryos were harvested at early (12th incubation day for chickens, 15th turkey/ducks) and late (19th chickens, 24th turkeys/ducks) incubation

stages. Virus growth in embryonic tissues was evaluated through histopathology, immunohistochemistry for nucleocapsid protein, and qRT-PCR for ABBV genome.
Results showed that ABBV replicated well in GEF, DEF, CCL-41 and QT35 cells, establishing a population of persistently infected cells. Growth in CEF, TEF and DF-1 cells was limited. Minimal virus replication was detected in embryonic tissues, suggesting that the *in ovo* model is not suitable to study ABBV pathogenesis.

A Preliminary Survey of Hemagglutinating Viruses from Penguins, Great Grebes and Petrels in the coast of the district of Pinamar, Argentina.

Celina Buscaglia Barreda[1,2, 3]

[1]Comision de Investigaciones Cientificas de la Provincia de Buenos Aires, Argentina
[2]Grupo Amantes de la Naturalea del Partido de Pinamar (GANPP). Arca de Noé 278, "Panchali", Pinamar 7167, Provincia de Buenos Aires, Argentina.
[3]Catedra de Zootecnia Especial III parte (Aves y Piliferos), Facultade de Cs Veterinarias, Universidad Nacional de La Plata, Argentina. During the first year

Avian Influenza (AI), an exotic disease, has never been detected serologically or isolated from suspicious cases where other causes were determined in poultry. Although Mohler in1926 indicated the presence of AI, it was impossible to diagnose neither it nor Newcastle disease in Argentina. In any case, it can**no**t be concluded that it was AI. In 2004 a public assignment was addressed by the director of the poultry section of the "Servicio Nacional de Sanidad y Calidad Agroalimentaria" (SENASA), to help on Avian Influenza Surveillance. This request was done during a meeting that took place at SENASA. However, these findings could not be published as was asked, but data were presented in two international meetings and a publication was done by only part of the researchers involved in the project plus a researcher that works in the US who was involved later. As a consequence, a new surveillance in wild birds was started in October 2008. Fecal samples obtained from penguins and petrels - Magallanic Penguins (*Spheniscus magellanicus*), Rockhopper Penguins (*Eudyptes chrysocome*) one King penguin (*Aptenodytes patagonicus*) and 2 Southern Giant Petrels (*Macronectes giganteus*) -,that arrived at the coast of the district of Pinamar were inoculated in 9 to11 day old embryonated eggs. After 5 days allantoic fluids were tested for evidence of hemoagglutination and two blind passages were performed. None of the samples were positive; only part of them were sent to SENASA where allantoic fluids from inoculated eggs were also tested by real time RT-PCR. Neither Avian Influenza viruses nor paramixoviruses were isolated and no avian influenza and/ or Newcastle disease viruses specific sequences were detected either.

Evaluation of Infectious Bronchitis Virus (IBV) Infection in Broilers Fed Availa®Zn

Brian S. Ladman[A], Lauren A. Sauble[A], Marcella M. Murphy[A], Erin M. Brannick[A], Jack Gelb, Jr.[A], and Marco A. Rebollo[B]

[A]Avian Biosciences Center, Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716
[B]Zinpro Corporation, Research & Nutritional Services, Eden Prairie, MN 55344

Infections bronchitis virus (IBV) is arguably the most difficult of all infectious diseases of chickens to control. The economic impact of IB on respiratory disease, mortality and airsacculitis condemnations has risen in recent years and it is likely to continue to do so given current production practices. Micronutrient supplements like Availa®-Zn (Zinpro Corporation, Eden Prairie, MN) fed to chickens can support immune health, thus ease the impact of disease, disease related losses and viral shedding. A Zinpro trace mineral feed supplement Availa®-Zn was added to a commercial broiler chicken starter feed at two concentrations to evaluate the effects on the innate immune system of a broiler chicken to prevent respiratory disease induced by IBV infection. After a 10 day pretreatment period, broilers were directly infected with a mildly pathogenic strain of IBV or placed in contact with infected broilers. From day 2-post inoculation (PI) to

day 10, all broilers were weighed and oropharyngeal (O/P) swabs were individually collected from all birds. Bodyweights, O/P swabbings and evaluation of clinical disease signs (coughing, tracheal rales, labored breathing) occurred daily from day 2 to day 9 PI. All birds were necropsied and all gross lesions (airsacculitis, pericarditis, perihepatitis) associated with respiratory disease were recorded. Birds fed Availa®Zn had greater daily weight gain during the post-challenge period as well as a decreased incidence of airsacculitis at necropsy. Moreover, indirectly infected birds (contact birds) consuming Availa-Zn at the high dose had decreased shedding of IBV. The results of this study support the hypothesis that zinc supplementation may decrease the biological and economic impact of respiratory disease by maintaining epithelial tissue health during a viral insult. § Prophylactic

Wealth of Knowledge

Non-biological factors impacting efficacy of Direct Fed Microbials in commercial poultry production

James T. Barton

Director of Technical Services, Adisseo, 3376 Peppermill Place, Fayetteville, Arkansas

Use of probiotics, direct fed microbials (DFMs), yeast subunits, and botanicals has become commonplace in an effort to satisfy the requests of consumers and customers for poultry products raised without antibiotics. Despite the common adoption of direct fed microbials in commercial poultry production, end users typically characterize their efficacy as inconsistent or unreliable. Discussions regarding efficacy of probiotics and DFMs typically focus on strain selection, CFU per kg of feed, and the ability to survive heat and pressure treatments (pelleting). Although these factors are important, it is equally important to consider factors that affect consistent distribution of viable organisms, germination rate, and quality assurance procedures. The presenter will describe formulation difficulties and innovative quality assurance procedures that may alleviate factors that drive inconsistency in the effects of DFMs.

Peptidoglycan: An issue in chicken intestinal tract that has been overlooked.

Tina Yun-Ting Wang

DSM Nutritional Products North America-Animal Nutrition and Health

Peptidoglycan (PGN) is the unique component in bacterial cell walls which is critical for bacteria survival. It makes up as much as 90% of Gram positive and 10% of Gram negative bacteria cell wall. The gastrointestinal tract is an abundant source of PGN because nearly 109-1011 cfu/g bacteria reside in chicken intestine. Research finds that approximately 50% PGN turnover occurs in one generation of bacteria resulting in free residual PGN in the intestinal tract. Free PGN or dead bacteria cell debris is hypothesized to interfere with digestive and absorptive processes, thereby lowering the efficiency of chicken performance. Muramidases are a well-known component of the natural defense systems of humans and animals. More than forty known muramidases exist with different specificity and function. We developed a novel muramidase from a natural source void of antimicrobial activity, but with a high specific activity to hydrolyze PGN and degrade only dead cell debris. In a 33-day floor pen study with 432 birds and 12 replicates/treatment, the muramidase group outperformed the control with 2% or 3 points improved feed conversion ($P<0.05$) and 2.5% higher body weight gain ($P<0.05$). In conclusion, feeding this novel muramidase significantly improved feed conversion and increased body weights presumably by increasing nutrient availability and absorption through the degradation of PGN and the cell wall debris from dead bacteria.

Toward Understanding the Respiratory Microbiota of Poultry

Chang-Won Lee, John M. Ngunjiri, Michael C. Abundo, Kara Taylor, Mohamed Elaish, Hyesun Jang, Hana Ji, Mahesh KC, Amir Ghorbani

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, United States of America
Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, United States of America

Complex microbial communities that occupy the respiratory system have a significant influence on the immune status and animal health. There is a need to define the baseline respiratory microbiota in order to facilitate studies that will illuminate the microbiome's role in host susceptibility and response to respiratory pathogens and vaccines. We first characterized the bacterial communities that were present in the upper and lower respiratory tract including sinus, trachea, air sacs and the lungs. In the second study, we evaluated field-utilized respiratory swab collection methods which produced extensive data where microbiome from tracheal swab and wash are distinct from each other while the tracheal and choanal swabs share a high degree of similarity. We also have sampled healthy flocks of commercial chickens and turkeys along the different phases of production. We found that age and body-site had the greatest impact on the bacterial microbiome of those birds. The core respiratory microbiota differed greatly from core gut microbiota in diversity and temporal dynamics, yet several members of the genus Lactobacillus were ubiquitous, suggesting a common function of this genus in both systems. We also showed that known avian pathogens can emerge and persist at subclinical levels in an optimally performing flock. Controlled experiments using SPF chickens are ongoing focusing on the effects of viral infections (influenza, reovirus, IBDV) and antiviral vaccines on commensal bacteria. Future work will establish core microbiome biomarkers of viral infections and the long-term impacts of acute viral infections on microbiome, and poultry performance.

Field Experiences in Preventing Bacterial Bone Disease in Broiler Chickens

Marie K. Severyn[a], Daniel A. Bautista[b], Mark A. Dekich[a], Erin M. Brannick[c],

[a]Amick Farms, LLC, Delmar, DE, USA
[b]University of Delaware, Lasher Laboratory, Georgetown, DE, USA
[c]University of Delaware, Department of Animal and Food Sciences, Newark, DE, USA

Bacterial bone diseases such as bacterial chondronecrosis with osteomyelitis (BCO) and "kinky-back" (spondylolisthesis) are major causes of lameness in broiler chickens. The causes of bacterial bone diseases may be related to rapid growth in affected birds and lack of a healthy microbiome and sufficient intestinal integrity. Clinical antibiotic treatment largely remains ineffective due to the location of infection as well as bacterial resistance patterns. Therefore, prevention is vital to successful management of these cases. The advent of probiotics and other natural products for use in poultry may be a viable solution for prevention of bacterial bone diseases. Poultry veterinary knowledge and experiences with products for field prevention and management techniques are necessary for successful control of these diseases.

Big Data in Poultry Production: Harnessing Information Regardless of Scale

Albert M. Payne1, William M. Payne[1]

[1]Simple Ag Solutions, Omaha, NE

In poultry, the massive amounts of data routinely generated is an often-underutilized asset that could bring improvements to both animal health and the business bottom line. Big Data, a combination of tools and strategies formerly the domain of tech giants, has seen increasing adoption in the agriculture industry. Seeking to improve operational processes, businesses of all sizes, including veterinary practices, have made efforts to apply

data to decisions As the regulatory environment and financial industries converge through data, three distinct issues emerge. The first is processing and converting large amounts of unstructured data from disparate sources into useful information. Second is regulatory compliance; the largest sources of information are used to ensure product quality and must adhere to government and industry standards. The third issue that continues to expand in importance is data security and access control as confidential information moves from internal ledgers and spreadsheets to cloud-based environments and platforms. This presentation begins with a discussion of the value of integrating Big Data tools and strategies to inform production processes. We address the topic of what constitutes a data management strategy, and how to implement industry best practices regardless of production scale. Compliance, recall, and general supply chain management are also presented as opportunities to use data to improve business operations. Finally, we discuss recent trends in the adoption of blockchain to enhance security, transparency, and authenticity.

Telemedicine applications for training purposes and for consultations under poultry field conditions.

Daniel Venne, Jean-Pierre Vaillancourt

Because of biosecurity concerns, it is getting more difficult to visit poultry farms. Distance between an interesting case and specialized expertise may also be long. These are two of several reasons why telemedicine could be a great tool for consulting, for learning, and even for research purposes. Using a relatively new software originally designed for human medicine (REACTs), we developed consulting and training sessions between veterinarians in Quebec and France. This poster would highlight the different features of this approach, including split screen, green background technology, data sharing, control sharing of files and session recording.

Incorporating the New NPIP SubPart J into the State's Plan.

Douglas Anderson

GPLN

The National Poultry Improvement Plan (NPIP) was started in 1935 as a Federal-State-Industry association focused on vertically transmitted diseases (Pullorum, MG, MS) and test standardization. The membership meets every two years to modify the rules for the efficiency of the group. In 2017, the NPIP met in Franklin, Tennessee. During the meeting, the attendees met and approved the addition of a new Subpart (Subpart J) dedicated specifically around the needs of the Upland Game Bird industry. In incorporating the Subpart into GA's plan and those of other Southeastern states, we have identified some small issues and the Subpart continues to develop to the needs of the Game Bird industry. Some of these issues and new ideas will be presented, as they may lay the ground work for other States to follow in the upcoming year.

Industry Strategies for Inclusion Body Hepatitis Control in Peru

Antonio Cobian[1]; Temis Soldevilla[1]; Virgilio Pacheco[1] & Francisco Perozo[2]

[1]Redondos, Huacho, Peru.
[2]Boehringer Ingelheim, Bogota. Colombia.

Inclusion body hepatitis (IBH) is one of the most important emerging diseases in poultry and Peru is endemic. The most prevalent serotypes currently circulating are 4, 8a, 7, 11, generating continuous outbreaks and economic losses. The approach for IBH control herein tested includes definitive diagnosis, cleaning, and biosecurity measures together with breeder and broiler vaccination. The vaccination program includes at least two serotype 4 tissue origin inactivated IBH vaccines for the breeders and killed (ND+IBH) hatchery vaccination of the offspring. This program has been successful during the last 4 years in 3.000.000 breeders and 337.400.000 broilers in controlling outbreaks. This

paper demonstrates a 100% drop in IBH cases and productive improvements (8.6% vs 3.12% mortality) in broilers within the program when compared with other vaccine strategies. Overall, this approach has shown effective cross-protection against the circulating serotypes, suggesting its suitability for IBH control under field conditions in Peru.

POULTRY HEALTH MANAGEMENT SCHOOLS: CONTINUING EDUCATION FOR THE POULTRY HEALTH PROFESSIONAL

Robert Porter[a], Richard Fulton[b], Darrin Karcher[c], Teresa Morishita[d], Ralph Stonerock[e]

[a]University of Minnesota, Veterinary Diagnostic Laboratory, St. Paul, MN,
[b]Michigan State University, Diagnostic Center for Population and Animal Health, East Lansing, MI,
[c] Purdue University, West Lafayette, IN,
[d]Western University, Pomona, CA,
[e]Circleville, OH

The Poultry Health Management School (PHMS), a non-profit, multi-state cooperative extension program, provides continuing education for poultry field service personnel and other avian health professionals. Nationally recognized course instructors, from university, industry, and government sectors, provide individualized training to course participants. The PHMS is composed of two separate schools: 1) Turkey and Broiler Health Management School and 2) Layer Health Management School. These schools consist of two days of intensive training consisting of didactic lectures and hands-on laboratory skills. Each year the schools have a different focus topic, i.e., vaccination/medication (2016), management and disease interactions (2017) and disease diagnostics (2018). Each school provides lecture and hands-on training in common diseases, diagnostics and how to manage a specific condition in the field. Day 1 provides information on basic anatomy, physiology and common diseases. Participants also learn basic skills in blood collection, euthanasia, performing a necropsy, vaccination and serological test interpretations. Day 2 allows the participants to put this information to use as they assess real disease cases, ending with current issues and opportunity for participants to ask questions in round table fashion. Participants receive a certificate of recognition upon completion of the program.

The effects of downtime on key chicken production parameters

Tim Buisker[a], Charles Corsiglia[b]

[a] Principal Data Scientist, Smart Data Science Solutions, 500 Hill St., Galena, IL 61036. [b] Manager of Veterinary Services, Foster Farms, 1000 Davis St., Livingston, CA 95334.

Our objective is to determine if a difference in downtime between bird placements is related to key production parameters of the subsequently placed flocks. This is a retrospective cohort analysis utilizing live operations and accounting data from 2009 commercial broiler flocks placed between January 2016 and October 2018. We analyze the following outcomes: average daily weight gain (ADWG), average final weight, week 1 mortality, livability, 6lb. adjusted feed conversion, total condemnations, and percent positive *Salmonella* boot sock tests. We then construct a machine-learning model using key inputs and outputs, as well as farm metadata, to predict cost-based effects specific to an individual producer's expenditures. Flocks with >= 18 days downtime prior to placement have statistically significantly higher average weight (6.12 vs. 5.98 lbs), ADWG (0.13 vs. 0.12 lbs), and livability (95.0% vs. 93.6%), and statistically significantly lower week 1 mortality (1.44% vs. 1.62%) and *Salmonella* boot sock prevalence (90% vs. 80%) compared to flocks with <=10 days downtime. The number of days of downtime between bird placements has statistically significant effects on multiple key production parameters. The effects vary by production parameter, and the machine-learning model indicates that the effects on the production parameters are dependent on the number of days of downtime. Combined with producer-specific expenditures, the model can be used to optimize production costs relative to production-based constraints.

Characterization of the Demographics and Prevalence of Poultry Pathogens in Ontario, Canada Small Poultry Flocks

Marina Brash[a], Durda Slavic[a], Davor Ojkic[a], Nancy Brochu[b], Csaba Varga[c], Michele Guerin[d], Brandon Lillie[b], Leonardo Susta[b]

[a]Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada. [b]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. [c]Ontario Ministry of Agriculture, Food and Rural Affairs, University of Guelph, Ontario, Canada. [d]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

The number of small poultry flocks being raised in Ontario has increased over the last decade. Despite this popularity, very little information on the husbandry, biosecurity practices and health status of these flocks is known. Between October 2015 and September 2017, the Animal Health Laboratory and the Pathobiology Department conducted a prospective study of small flock submissions, which included full postmortem analysis and a pre-set panel of microbiological tests. Participating owners signed a consent form and completed a husbandry and biosecurity questionnaire. A total of 160 submissions were received with chickens most commonly submitted (82 %), followed by turkeys, game birds, and ducks. Pre-set microbiological tests detected *Campylobacter* spp., *Brachyspira* spp., *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, and *Salmonella* spp. in 35, 37, 36, 23, and 3% of tested submissions. Infectious bronchitis virus, fowl adenovirus, infectious laryngotracheitis virus, reovirus and infectious bursal disease virus were detected in 39, 35, 15, 4, and 1 % of submissions. Low path avian influenza virus (H10N8) was detected in one turkey and vaccine strain avian avulavirus-1 from one chicken submission. No avian bornavirus was detected. Birds were most commonly raised for self-consumption of meat and/or eggs. Over 33% of birds were housed in mixed groups with different species and/or different commodities in the same coop and 66% of birds had outside access. This study provides baseline data about flock disease prevalence and demographic characteristics, which will aid in assessing the risks that these flocks may pose to commercial poultry and in developing extension publications.

Causes of Morbidity and Mortality in Small Flocks in Ontario

Leonardo Susta[a], Nancy Brochu[a], Michele Guerin[b], Csaba Varga[c], Brandon Lillie[a], Marina Brash[d].

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph (Ontario), N1G 2W1, Canada. [b]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph (Ontario), N1G 2W1, Canada. [c]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph (Ontario), N1G 2W1, Canada. [d]Animal Health Laboratory, Guelph (Ontario), N1G 2W1, Canada.

Non-commercial poultry flocks (referred to as "small flocks") have become increasingly popular in Canada during the recent years. Little is known about the main causes of morbidity and mortality (health status) that affect these flocks. In this study, the baseline prevalence of common infectious and non-infectious diseases among Ontario's small poultry flocks was assessed by conducting a prospective surveillance study over a 2-year period. With the owner's consent, for each bird submitted to the Animal Health Laboratory we performed a full postmortem exam, including ancillary tests to reach a final diagnosis. A total of 245 birds from 160 submissions were necropsied and tested for pathogens. Infectious diseases were the most common primary cause of clinical signs or death (62%) among all submitted birds. Multifactorial respiratory diseases (21%) and Marek's disease (11%) were the most common infectious primary causes for death or
reported clinical signs. Mixed respiratory infections were caused by various combinations of infectious laryngotracheitis and infectious bronchitis virus, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *E. coli*, *Avibacterium* spp. and *Gallibacterium* spp.

No federally reportable diseases were diagnosed. Disturbances of growth were the cause of clinical signs or death in 27 birds (11%). Physical etiologies, such as predation, cannibalism, and other trauma-related injuries caused death in 8 chickens. The health status of small flocks in Ontario has not been reported previously, and these data will provide helpful baseline information for knowledge transfer materials directed to owners and veterinarians, and ultimately aid in the prevention and control of diseases among small poultry flocks.

Review and statistical analysis of trends in common diseases of pet/hobby chickens submitted to the Georgia Poultry Lab Network

Christopher Gaudette, BS[a]; Doug Anderson, DVM[b]; James Davis, DVM[b]

[a]North Carolina State University, Raleigh, North Carolina;
[b]Georgia Poultry Lab Network, Gainesville, Georgia

The number of pet/hobby chicken cases submitted to the Georgia Poultry Lab Network have increased from 14% of cases in 2014 to 18% of cases in 2017. This trend makes diagnostic work by small animal veterinarians and poultry industry professionals of increasing importance for disease prevention in both sectors. The diagnosis of the cases from 2013 through 2018 will be analyzed and presented for future protocol and prevention. Of the 96 cases presented from 2013 through 2014, 31% were diagnosed Marek's disease with intestinal parasites second. Results on the change in pet/hobby chicken disease over the last 4 years, at the Georgia Poultry Lab Network, will be presented.

Mycotoxin Prevalence in the 2018 Latin American Corn Crop

Eduardo Vicuña*[1], Alexandro Marchioro2

[1]BIOMIN Brasil, Piracicaba - SP, Brasil
[2]BIOMIN Holding GmbH, Getzersdorf, Austria

Commercial harvests may be contaminated by mycotoxins, toxic secondary metabolites produced by different fungal species. Fungus can be classified into two species, the ones producing metabolites on the field (e.g. Fusarium spp.) and the ones on storage (e.g. Aspergillus and Penicillium spp.). Mycotoxin assessment of animal feed is important to determine the risk to animal health and performance. BIOMIN maintained a global survey of mycotoxin contamination since 2004. A total of 2074 corn samples were collected from Latin America (including Mexico) and analyzed from January to September 2018; the methods used were ELISA and LC-MS/MS. The six major mycotoxin groups analyzed comprised of aflatoxins, type A trichothecenes such as T-2 toxin, type B trichothecenes such as deoxynivalenol (DON; vomitoxin), ochratoxin-A, fumonisins (FUM), and zearalenone (ZEN) derivatives. Maximum field contamination levels (in µgkg-1) found are considered an important challenge (Afla: 402; ZEN: 5020; DON: 6000; T2: 293; FUM: 35840; OTA: 75). Trends in specific countries and particularly in Argentina and Brazil are discussed. The preliminary results from the 2018 Latin American corn harvest suggest the mycotoxins contamination levels represent a risk for poultry. This includes contamination with FUM, DON, and ZEN. Because of the high frequency of multi-mycotoxin contamination in samples thus far, multiple strategies of mitigating risk are needed beyond adsorption, including biotransformation and providing support to immune and liver function.

Megabacteriosis, *Macrorhabdus ornithogaster* and *Mycobacterium genavense* in a Zoo Budgerigar, *Melopsittacus undulatus*, Flock

Richard M. Fulton and Rinosh Mani

Michigan State University Veterinary Diagnostic Laboratory and Pathobiology and Diagnostic Investigation College of Veterinary Medicine Michigan State University East Lansing, Michigan 48824

Animals that die in zoological collections are typically sent to diagnostic laboratories for diagnosis as part of a routine monitoring program. Over a four month period, a Michigan zoo had thirteen budgerigars, *Melopsittacus undulatus,* from their quarantine flock die and submitted to the diagnostic

laboratory for analysis. Three of them died from avian tuberculosis. Mycobacterium was cultured and identified by DNA sequence to be *Mycobacterium genavense*. In the other two birds, the acid fast bacilli were not able to be further identified. Of the affected birds, two had splenomegaly. Acid fast bacteria were detected in the spleen of only one bird. The other bird with splenomegaly had acid fast bacilli only in the intestine. The third bird had acid fast bacteria in the lungs. During that same time period, four budgerigars were determined to have avian gastric yeast due to *Macrorhabdus ornithogaster*. Historically, this infection has been termed megabacteriosis. All four birds were emaciated with atrophy of breast musculature and decreased subcutaneous and coelomic fat stores. Only one bird had proventricular dilatation. Microscopic exam of the proventriculus of all birds revealed the presence of a myriad of 4 x 50 µm septate yeast organisms that were morphologically consistent with *Macrorhabdus ornithogaster.* The remainder of the budgerigars died from exclusion by flock mates, nephrosis and oviductal prolapse with an adhered egg.

A Historical Snapshot of Turkey Production in the USA

Joan S. Schrader, DVM, MS, ACPV

Veterinary Consultant, Ashland, NE 68003

In the years between 1925 and 1955 turkey production in the USA was broadly distributed across the United States. Extension Services in at least 10 states published and distributed publications to aid new turkey growers in brooding, housing, nutrition and disease control. Multiple Universities published research to advance techniques in breeding by artificial insemination, incubation and hatching and disease control. Numerous allied industries sold treatments containing herbs and beneficial bacteria in a time before antibiotics. Major turkey diseases of the time will be compared and contrasted with today's situation.