▶**AAAP Symposium**
Emerging and Reemerging Zoonotic Diseases

▶ **AAAP Scientific Program**
includes 148 oral presentations
and 82 poster presentations

AUGUST 6-9 | 2016

AAAP
Symposium & Scientific Program

THE AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS

promotes scientific knowledge to enhance the health, well-being, and productivity of poultry to provide safe and abundant food for the world.

San Antonio, TX
August 6-9, 2016

AAAP 2016 Annual Meeting
San Antonio Convention Center
San Antonio, Texaz, USA August 6-9, 2016

Saturday, August 6, 2016 (7:30AM – 12:30PM)
AAAP Symposium
Emerging and Reemerging Zoonotic Diseases
San Antonio Convention Center, Room 221AB

Saturday, August 6, 2016 (2:00PM – 5:00PM)
AAAP Committee Meetings
Marriot River Center Hotel

Saturday, August 6, 2016 (5:00PM – 7:00PM)
AAAP New Member Meet and Greet
AAAP Members are invited to welcome new members
Marriot River Center Hotel, Salon I

Sunday-Tuesday, August 7-9, 2016 (8:00AM – 5:30PM)
AAAP Scientific Program
San Antonio Convention Center
Sessions A: Room 221AB
Sessions B: Room 221CD
Poster Room: 220

Sunday, August 7, 2016 (9:30AM – 9:45AM)
Reed Rumsey Basic Research Award Winner: **Correlation between Interferon Response and Protective Efficacy of NS1-Truncated Mutants as Influenza Vaccine Candidates in Chickens**
Hyesun Jang
The Ohio State University
San Antonio Convention Center, Room 221CD

Sunday, August 7, 2016 (11:00AM – 11:30AM)
Keynote Speaker: **Emerging and Reemerging Poultry Diseases: An International Perspective**
Guillermo Zavala
Avian Health International, LLC, Flowery Branch, GA
San Antonio Convention Center, Room 221AB

Sunday, August 7, 2016 (11:30AM – 12:30PM)
AAAP Business Meeting
San Antonio Convention Center, Room 221AB

Monday, August 8, 2016 (11:15AM – 11:45AM)
Lasher-Eckroade History Lecture: **The History of the First Generation Marek's Disease Vaccines: The Science and Little Known Facts**
Karel Schat
Cornell University, Ithaca, NY
San Antonio Convention Center, Room 221AB

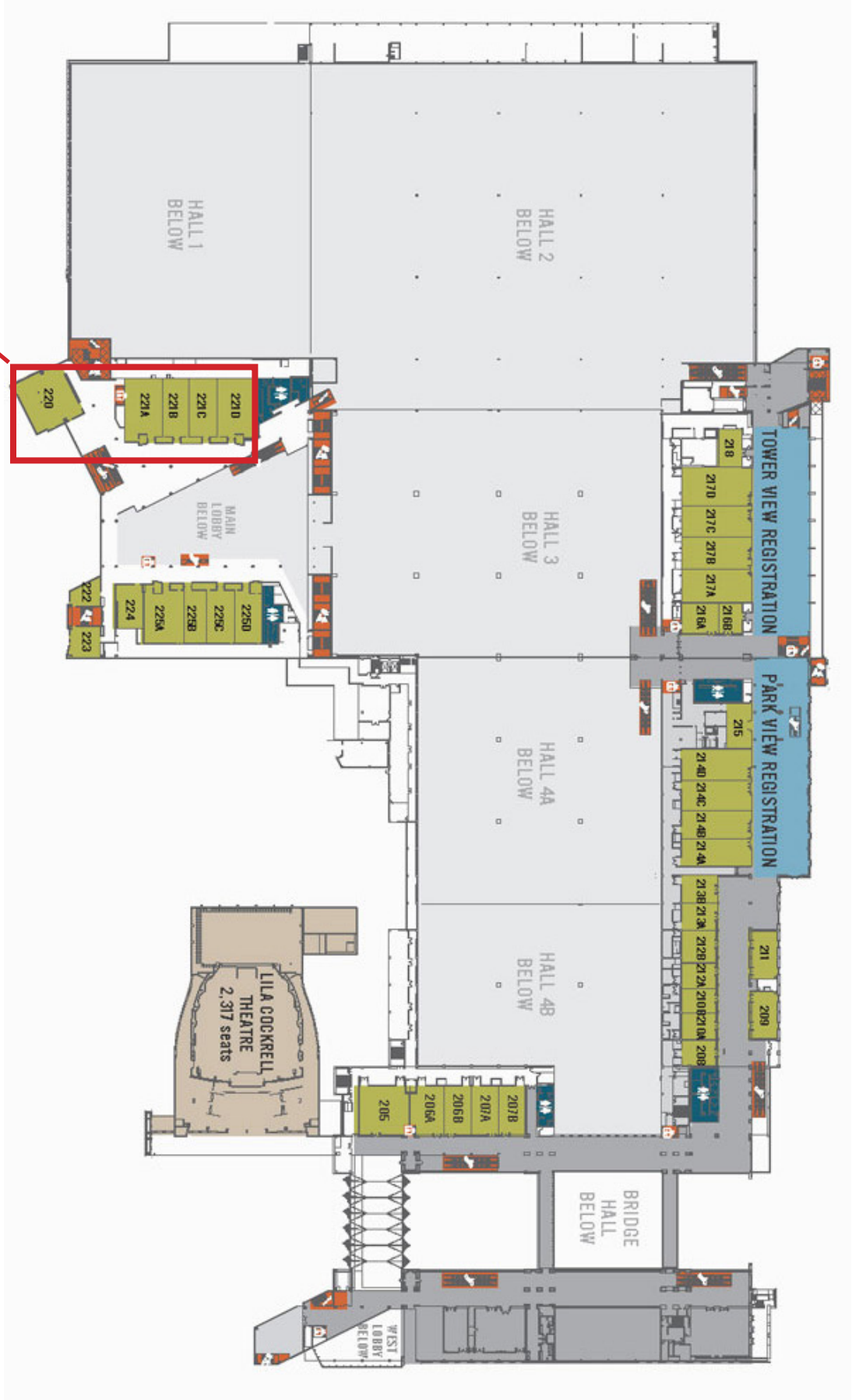
Monday, August 8, 2016 (12:00PM – 2:00PM)
AAAP Awards Luncheon
Marriot River Center Hotel, Salon E

Monday, August 8, 2016 (4:30PM – 5:30PM)
Wine & Cheese Social in Poster Room
Poster Presenters will be present at their posters during this time.
San Antonio Convention Center, Room 220

Tuesday, August 9, 2016 (8:15AM – 8:30AM)
Richard B. Rimler Memorial Paper: **Evaluation of Viral and Host Mechanisms Involved in Permanent Marek's Disease Virus Induced Immunosuppression**
Faiz Nik Mohd Azmi
North Carolina State University
San Antonio Convention Center, Room 221AB

Tuesday, August 9, 2016 (1:15PM – 1:30PM)
Reed Rumsey Clinical Research Award Winner: **Study on the Prevalence and Association of a Novel Mycoplasma sp. with Reproductive Disease in Commercial Goose Breeders.**
Silvia Carnaccini
CAHFS-UCDavis
San Antonio Convention Center, Room 221CD

San Antonio Convention Center
Meeting Level 3



2016 AAAP Event Schedule

Name of Group	Meeting Date	Beg. Time	End Time	Contact Person	Room	Location
AAAP Board of Directors Meetings						
AAAP Board of Directors	Thursday, Aug 4	8:00am	5:00pm	Janece Bevans-Kerr	Conference Room 9	Marriott
AAAP Board of Directors	Friday, Aug 5	7:00am	5:00pm	Janece Bevans-Kerr	Conference Room 9	Marriott
AAAP Board of Directors	Tuesday, Aug 9	7:00am	12:00pm	Janece Bevans-Kerr	Conference Room 17&18	Marriott
Committee Meetings						
Media Training	Friday, Aug 5	1:00pm	5:00pm	Janece Bevans-Kerr	Conference Room 15	Marriott
Histopathology/Case Report Interest Group	Friday, Aug 5	1:00pm	5:00pm	H.L. Shivaprasad	Conference Room 12	Marriott
Backyard Flocks Interest Group	Friday, Aug 5	4:00pm	5:00pm	Vicky Bowes	Conference Room 10	Marriott
Committee Review Committee	Friday, Aug 5	5:00pm	6:00pm	Bruce Stewart-Brown	Conference Room 19	Marriott
Poultry Scholarship Committee	Saturday, Aug 6	6:30am	7:00am	Mark Bland	Conference 5	Marriott
AAAP Committee Chairs and BOD Meeting	Saturday, Aug 6	12:45pm	2:00pm	Suzanne Dougherty	Conference Room 17&18	Marriott
Awards Committee	Saturday, Aug 6	2:00pm	3:00pm	Kate Barger	Conference Room 11	Marriott
Avian Diseases Editorial Board	Saturday, Aug 6	2:00pm	3:00pm	Mo Saif	Conference Room 1-4	Marriott
Avian Diseases Manual Editorial Board	Saturday, Aug 6	2:00pm	3:00pm	Martine Boulianne	Conference Room 19	Marriott
Diseases of Public Health Significance	Saturday, Aug 6	2:00pm	3:00pm	Bill Pierson	Conference Room 9	Marriott
Enteric Diseases Committee	Saturday, Aug 6	2:00pm	3:00pm	Marco Quiroz	Conference Room 15	Marriott
LAC Committee	Saturday, Aug 6	2:00pm	3:00pm	Suzanne Dougherty	Conference Room 10	Marriott
Preceptorship Committee	Saturday, Aug 6	2:00pm	3:00pm	Francene Van Sambeek	Conference Room 16	Marriott
Toxic, Infectious, Miscellaneous & Emerging Diseases Committee	Saturday, Aug 6	2:00pm	3:00pm	Sunil Mor	Conference Room 17&18	Marriott
Drugs and Therapeutics Committee	Saturday, Aug 6	3:00pm	4:00pm	Steven Clark	Conference Room 16	Marriott
Education Committee	Saturday, Aug 6	3:00pm	4:00pm	Gabriel Senties-Cue	Conference Room 8	Marriott
Membership Committee	Saturday, Aug 6	3:00pm	4:00pm	Deirdre Johnson	Conference Room 6	Marriott
Avian Diseases Advisory Board	Saturday, Aug 6	3:00pm	4:00pm	Mo Saif	Conference Room 5	Marriott
Tumor Virus Committee	Saturday, Aug 6	3:00pm	5:00pm	Isabel Gimeno	Conference Room 12	Marriott
Animal Welfare Committee	Saturday, Aug 6	3:00pm	5:00pm	Rosemary Marusak	Conference Room 1-4	Marriott
Research Priorities Committee	Saturday, Aug 6	3:00pm	4:00pm	Ivan Alvarado	Conference Room 11	Marriott
Respiratory Diseases Committee	Saturday, Aug 6	3:00pm	5:00pm	Mark Jackwood	Conference Room 17 &18	Marriott
Epidemiology Committee	Saturday, Aug 6	4:00pm	5:00pm	J. P. Vaillancourt	Conference Room 6	Marriott
Food Safety Committee	Saturday, Aug 6	4:00pm	5:00pm	James Barton	Conference Room 9	Marriott
History Committee	Saturday, Aug 6	4:00pm	5:00pm	John Dunn	Conference Room 13 & 14	Marriott
AAAP Foundation Development Committee	Monday, Aug 8	8:00am	9:00am	Fred Hoerr	Salon C	Marriott

2016 AAAP Event Schedule (continued)

Name of Group	Meeting Date	Beg. Time	End Time	Contact Person	Room	Location
Program Events						
Stakeholder Meeting with CVB	Friday, Aug 5	3:00pm	5:00pm	Janece Bevans-Kerr	Conference Room 1-4	Marriott
AAAP Symposium	Saturday, Aug 6	7:30am	12:30pm	Janece Bevans-Kerr	221AB	Convention Center
New Member Orientation	Saturday, Aug 6	4:30pm	5:00pm	Janece Bevans-Kerr	Conference Room 8	Marriott
Meet and Greet New AAAP Members	Saturday, Aug 6	5:00pm	6:00pm	Janece Bevans-Kerr	Salon I	Marriott
AAAP Business Meeting	Sunday, Aug 7	11:30am	12:30pm	Janece Bevans-Kerr	221AB	Convention Center
AAAP Awards Luncheon	Monday, Aug 8	12:00pm	2:00pm	Janece Bevans-Kerr	Salon E	Marriott
AAAP Wine and Cheese Social in Poster Room	Monday, Aug 8	4:30pm	5:30pm	Janece Bevans-Kerr	220	Convention Center
ACPV						
ACPV Exam #1	Friday, Aug 5	7:00am	8:00pm	Janece Bevans-Kerr	Conference Room 13 & 14	Marriott
ACPV Exam #2	Friday, Aug 5	7:00am	8:00pm	Janece Bevans-Kerr	Salon K & L	Marriott
ACPV Exam Grading Room	Friday, Aug 5	7:00am	10:00pm	Janece Bevans-Kerr	Conference Room 11	Marriott
ACPV Board of Governors Meeting	Sunday, Aug 7	6:30am	10:30am	Janece Bevans-Kerr	Salon C	Marriott
ACPV Reception/Annual Meeting	Monday, Aug 8	6:30am	8:00am	Janece Bevans-Kerr	Conference Room 1-4	Marriott
Invitation Only						
Association of Veterinarians in Broiler Production Meeting	Friday, Aug 5	7:00am	5:00pm	Deirdre I Johnson	Conference Room 17&18	Marriott
Association of Veterinarians in Broiler Production Breakfast	Friday, Aug 5	7:00am	9:00am	Deirdre I Johnson	Conference Room 16	Marriott
Association of Veterinarians in Broiler Production Lunch	Friday, Aug 5	1:00pm	2:00pm	Deirdre I Johnson	Conference Room 16	Marriott
Association of Veterinarians in Turkey Production	Friday, Aug 5	9:00am	5:00pm	Duane Murphy	Salon A	Marriott
Georgia MAM Alumni Breakfast	Saturday, Aug 6	7:00am	7:30am	Karen Grogan	Conference Room 1-3	Marriott
Association of Poultry Primary Breeder Veterinarians	Saturday, Aug 6	11:30am	1:30pm	Travis Schaal	Conference Room 5	Marriott
Association of Veterinarians in Egg Production	Sunday, Aug 7	6:00am	8:00am	Eric Gingerich	Conference Room 17&18	Marriott
AAAP Past Presidents Luncheon	Sunday, Aug 7	12:15pm	1:45pm	Janece Bevans-Kerr	Conference Room 4	Marriott
NC State University Poultry Health Management	Monday, Aug 8	7:30pm	11:00pm	Michael Martin	Conference Room 17	Marriott

2015-2016 AAAP Foundation Board of Directors

www.aaap.info/foundation

Frederic J. Hoerr
President

Robert L. Owen
Past President

Robert E. Porter
AAAP President 2016

Hector Cervantes
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Past President 2016

AAAP Representatives to AVMA

AVMA Animal Agriculture Liaison
Karen Grogan 2018
Mark Burleson 2018 (Alternate)

AVMA Animal Welfare Committee
Michael P. Martin 2016
Kate Barger 2016 (Alternate)

AVMA Clinical Practitioners Advisory Committee
Hector M. Cervantes 2017
Chad Malinak 2018 (Alternate)

AVMA Committee for Disaster and Emergency Issues
Jarra Jagne 2016

AVMA Convention Education Program Committee
Danny L. Magee 2018

AVMA Food Safety Advisory Committee
Eric Willinghan 2018
Charles L. Hofacre 2016 (Alternate)

AVMA House of Delegates
Y. M. Saif 2016
Gregg J. Cutler 2016 (Alternate)

AVMA Legislative Advisory Committee
Suzanne Y. Dougherty 2018
Bruce Stewart-Brown 2016 (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 2018
David R. Hermes 2017

United States Animal Health Association (USAHA)
Eric Gingerich 2016

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

Animal Agriculture Coalition (AAC)
Ian Rubinoff

US Stakeholder Forum on Antimicrobial Resistances(S-FAR)
Randy Singer

AAAP Foundation Committee Chairs

www.aaap.info/aaapawards

Awards
Kate Barger 2016

Kenneth Eskelund Preceptorship
Francene S. Van Sambeek

Poultry Scholarship
Mark Bland

AAAP Constitutional Committee Chairs

www.aaap.info/committees

Auditing	Nominating	Resolutions
Karen Burns Grogan	Patricia A. Dunn	Frederic J. Hoerr

AAAP Task Force Committee Chairs

Animal Welfare and Management Practices
Rosemary A. Marusak 2016

Diseases of Public Health Significance
Frank W. Pierson 2016

Drugs and Therapeutics
Steven Clark 2016

Education
Patricia S. Wakenell 2016

Enteric Diseases
Marco Quiroz 2016

Epidemiology
Jean-Pierre Vaillancourt 2016

Food Safety
James T. Barton 2016

History of Avian Medicine
John R. Dunn 2016

Legislative Advisory
Suzanne Y. Dougherty 2018

Membership
Deirdre Ida Johnson 2016

Program Advisory
Danny Magee 2018
Ivan Alvarado, Vice Chair 2018

Respiratory Diseases
Mark Jackwood 2017

Research Priorities
Ivan R. Alvarado 2018

Tumor Virus
Isabel M. Gimeno 2018

Toxic, Infectious, Miscellaneous and Emerging Diseases
Sunil Mor 2019

AAAP Interest Group Chairs

Histopathology/Case Report Interest Group
H.L. Shivaprasad

2015-2016 AAAP Board of Directors

www.aaap.info/aaap-board-of-directors

Robert E. Porter
President 2016

Hector Cervantes
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Charles L. Hofacre
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Eva Wallner-Pendleton
Director - Northeast 2017

Eric L. Jensen
Director - South 2018

Bernard J. Beckman
Director - Central 2019

Victoria A. Bowes
Director - Western 2016

Ian Rubinoff
Director-at-Large 2016

Martine Boulianne
Director-at-Large 2017

Francene Van Sambeek
Past President 2016

AAAP Publication Board Chairs

Avian Diseases Advisory Board
Y. M. Saif

Avian Diseases Editorial Board
Y. M. Saif

Avian Diseases Manual
Martine Boulianne

Avian Histopathology Manual Board
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

AAAP Symposium

Emerging and Re-emerging Zoonotic Diseases

Saturday, August 6, 2016 (7:30AM – 12:30PM)
Room 221AB

7:30- 7:40 AM	**Introduction & Welcome** Frank (Bill) Pierson
Moderators	Nathaniel Tablante
7:40- 8:30 AM	**Consumer Perspectives on the Safety of Poultry Products** Robert O-Connor, *Foster Farms, Livingston, CA*
8:30- 9:00 AM	**Practical Methods of AI Prevention and Control to Protect Poultry and Humans** Jack Shere, *USDA-APHIS, Riverdale, MD*
9:00- 9:30 AM	**The Interface Between Public Health and Commercial Poultry Production: A View of Things to Come** Frank (Bill) Pierson, *Virginia Tech, Check, VA*
9:30- 10:00 AM	**Recent Avian Influenza Outbreaks in Poultry and Humans: An Epidemiologic Perspective** David Swayne, *USDA/ARS/SEPRL, Athens, GA*
10:00- 10:15 AM	**Questions and Answers for the First Four Talks**
10:15- 10:30 AM	Break
Moderators	Frank (Bill) Pierson
10:30- 11:10 AM	**How Epidemiology is Used in Investigating Poultry Disease Outbreaks** Jean-Pierre Vaillancourt, *University of Montreal, St. Hyacinthe, Canada*
11:10 AM - 11:30 AM	**Communicating Science-Based Information to Non-Scientists** Nathaniel Tablante, *University of Maryland, College Park, MD*
11:30 AM - 12:10 PM	**How Poultry Veterinarians can be More Involved in the "One Health Initiative"** Cheryl Stroud, *Executive Director, One Health Commission, Apex, NC*
12:10- 12:30 PM	**Questions and Answers, Session Wrap Up**
12:30 PM	Adjourn

AAAP Scientific Program
Sunday, August 7, 2016

	Room 221 AB	Room 221 CD
Topic	Case Reports	Avian Influenza
Moderator	Travis Cigainero	Louis Dufour-Zavala
8:00 AM	**Investigation of Egg Production Drops in Commercial Brown Layers: Part 1** Kelli Jones, *CEVA Animal Health, Yazoo City, MS.*	**A Microfluidic Device Integrated with an Advanced Nanostructured Material for Rapid Avian Influenza Virus Capture and Detection** Yin-Ting Yeh, *The Pennsylvania State University, University Park, PA.*
8:15 AM	**Egg Production Drops in Commercial Brown Layers Investigated: Part 2** Eva Wallner-Pendleton, *Pennsylvania State University, University Park, PA.*	**Transmission of Recent H5 Highly Pathogenic Avian Influenza Viruses in Chickens** David Suarez, *Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA.*
8:30 AM	**Investigation of Egg Production Drops in Commercial Brown Layers: Part 3** Milos Markis, *AviServe LLC, Newark, DE.*	**Characterization of H9N2 Low Pathogenic Avian Influenza Viruses from Pakistan (2012-2015)** Dong-Hun Lee, *Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA.*
8:45 AM	**A Unique Case of Ulcerative Dermatitis in a Flock of Cage Free, Commercial Brown Layers** Geoffrey Lossie, *Indiana ADDL, Lafayette, IN.*	**The Multigenic Nature of the Differences in Pathogenicity of H5N1 Highly Pathogenic Avian Influenza Viruses in Domestic Ducks** Mary Pantin-Jackwood, *Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA.*
9:00 AM	**Bone Deformities in Roller Pigeon Squab due to Improper Diet.** Susan Williams, *University of Georgia, PDRC, Athens, GA.*	**Update on Avian Influenza in the US.** Mia Torchetti, *USDA APHIS, Ames, IA.*
9:15 AM	**Break**	
Topic	Case Reports (continued)	Avian Influenza (continued)
Moderator	Enrique Montiel	Natalie Armour
9:30 AM	**Calcium Tetany in Flocks of Commercial Male Turkeys?** H Shivaprasad, *University of California, Davis, Tulare, CA.*	**Reed Rumsey Award Winner** * **Correlation between Interferon Response and Protective Efficacy of NS1-Truncated Mutants as Influenza Vaccine Candidates in Chickens** Hyesun Jang, *The Ohio State University, Wooster, OH.*
9:45 AM	**Leucocytozoon sp. in a Flock of 4-Week-Old Ducklings** Richard Fulton, *Michigan State University, Lansing, MI.*	**Peptide Nanoparticle-based Vaccine Protects Chickens against High Pathogenicity Avian Influenza Virus** Mazhar Khan, *University of Connecticut, Storrs, CT.*
10:00 AM	**Impact of Astrovirus Challenge on a Commercial Broiler Breeder Flock and Subsequent Progeny** David French, *Sanderson Farms, Laurel, MS.*	**Development and Application of a Vaccination Planning Tool for Avian Influenza** David Castellan, *FAO (past), Consultant, Niagara Falls, Canada.*
10:15AM	**Incursion and Recursion of "White Chicks" in U.S. Commercial Broiler Production** Philip Stayer, *Sanderson Farms, Laurel, MS.*	**Inactivated Vaccine Protects Layer Hens from Clade 2.3.4.4 H5 High Pathogenicity Avian Influenza Virus** Kateri Bertran Dols, *Southeast Poultry Research Laboratory, USDA-ARS, Athens, GA.*
10:30 AM	**Eimeria mivati: Field Clinical Case** Andres Montoya, *Merck Animal Health, Roswell, GA.*	**Higher Quantity of H5N2 Clade 2.3.4.4 High Pathogenicity Avian Influenza Virus Required to Infect Broilers than Leghorns but No Difference in Age Susceptibility** David Swayne, *USDA/ARS/SEPRL, Athens, GA.*
10:45 AM	**Break**	
Moderator	Danny Magee	
11:00 AM	Keynote Speaker: **Emerging and Reemerging Poultry Diseases: An International Perspective** Guillermo Zavala, *Avian Health International, LLC, Flowery Branch, GA* **Room 221AB**	
11:30 AM	**AAAP Business Meeting** **Room 221AB**	
12:30 PM	Adjourn for Lunch	
Topic	Case Reports (continued)	Avian Influenza (continued)
Moderator	Chad Malinak	Mary Pantin-Jackwood
1:30 PM	**Nasal Gland Chlamydiosis in Commercial Organic Turkeys in California: Association with Pigeons and Chlamydia psittaci Strain Characterization.** Gabriel Sentíes-Cué, *CAHFS-Turlock/UC Davis, Turlock, CA.*	**Network Modeling to Predict Avian Influenza Immunity Distribution in the Poultry Industry in Vietnam and Bangladesh** Fernando Lozano, *CIRAD- AGIRs, Libourne, France.*

Sunday, August 7, 2016 (continued)

	Room 221 AB	Room 221 CD
1:45 PM	**Sep-Tox: Condemnation Without Representation** Armando Mirandé, *Supervet, Inc., Montgomery, TX.*	**A Simulation Based Evaluation of the Time to Detect EA/NA H5N2 HPAI Virus Infection in Commercial Turkey Flocks under Various Active Surveillance Testing Protocols.** Carol Cardona,
2:00PM	**Ochroconosis and Rickets in Commercial Broiler Chicks** Natalie Armour, *Mississippi State University, CVM, DPPM, Pearl, MS.*	**Avian Influenza Outbreak Data Management System (British Columbia)** Victoria Bowes, *Animal Health Centre, BC Ministry of Agriculture, Abbotsford, Canada.*
2:15PM	**Nutrition-Induced Respiratory Disease in Turkeys** Kabel M. Robbins, *Butterball, LLC, Ozark, AR.*	**Pathogenicity of 2015 North American H5N2 Highly Pathogenic Avian Influenza Poultry Isolates in Chickens and Mallards** Eric DeJesus, *Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA.*
2:30 PM	**Histopathology of Nutrition-Induced Respiratory Lesions in Turkeys** Oscar Fletcher, *North Carolina State University, Raleigh, NC.*	**Investigation on the Possible Application of a Serological DIVA Monitoring Strategy when a rHVT-H5 Vaccine is used to Control Avian Influenza.** Yannick Gardin, *CEVA Santé Animale, Libourne, France.*
2:45 PM	**Variation in Gross and Histologic Lesions in Two Field Cases of Clade 2.3.4.4 H5 HPAI in British Columbia, Canada** Victoria Bowes, *Animal Health Centre, BC Ministry of Agriculture, Abbotsford, Canada.*	**Heterosubtypic Immunity to Low Pathogenic Avian Influenza Viruses in Mallard Ducks** Karen Segovia, *The University of Georgia, Athens, GA.*
3:00 PM	**Pathology Associated with Highly Pathogenic H5N8 Avian Influenza in Commercial Chickens and Turkeys in California** Simone Stoute, *UC Davis, Turlock, CA.*	**Preventing Outbreaks of Avian Influenza Through Timely Dissemination of Practical Science-Based Information** Nathaniel Tablante, *University of Maryland College Park, College Park, MD.*
3:15 PM	**Break**	
Topic	Case Reports (continued)	Avian Influenza (continued)
Moderator	Don Ritter	Mohamed El-Gazzar
3:30 PM	**Predation of a Flock of Mallard Cross Ducklings** Richard Fulton, *Michigan State University, Lansing, MI.*	**Inactivation of Avian Influenza Virus In Chicken Feed** Haroldo Toro, *Auburn University, Auburn, AL.*
3:45 PM	**Investigation on the use of a Serological DIVA Monitoring Strategy when a rHVT-F Vaccine is used to Control Newcastle Disease.** Yannick Gardin, *CEVA Santé Animale, Libourne, France*	**Inactivation of Avian Influenza Virus in Chicken Litter as a Potential Method to Decontaminate Poultry Houses** Christopher Stephens, *SEPRL-USDA-ARS, Athens, GA.*
Topic	Enteric Health	Virology
Moderator	Don Ritter	Mohamed El-Gazzar
4:00 PM	**A New Enteric Disease in Minnesota Turkeys caused by Picorna- and Picobirnaviruses?** Sunil Kumar, *University of Minnesota, Saint Paul, MN.*	**Mutation of Avian Encephalomyelitis Virus during Embryo Adaption** Rüdiger Hauck, *University of California, Davis, Davis, CA.*
4:15 PM	**Phylogenetic Analyses of Diverse, Novel Enteric Picornaviruses Detected in Turkeys and Chickens in the United States.** Michael Day, *USDA/ARS, Athens, GA.*	**Virological and Molecular Characterization of Chicken Astrovirus Associated with Reduction in Hatchability, Poor Chick Quality and "White Chick Disease."** Alejandro Banda, *Mississippi State University, Pearl, MS.*
4:30 PM	**Prevalence of Parvovirus in Minnesota Turkeys** Tamer Sharafeldin, *University of Minnesota, Saint Paul, MN.*	**White Chick Syndrome in Ontario: Clinical Features, Pathology and Viral Etiology** Emily Martin, *University of Guelpha, Breslau, Canada.*
Topic	Enteric Health (continued)	Infectious Bursal Disease
4:45 PM	**Does Early Exposure to Clostridium perfringens Provide some Immunity to a Severe Clostridium perfringens Oral Challenge at 17 Days in a Necrotic Enteritis Challenge Model?** Stephen Davis, *Colorado Quality Research, Inc., Wellington, CO.*	**Determination of Baseline for Bursa: Body Weight Ratio in Broilers in Relation to Sex & Breed over Age by using HTSi Data Package.** Manjunatha Mahabalarao, *Elanco Animal Health, Waxhaw, NC.*
5:00 PM	**Necrotic Enteritis in Turkeys in California and Characterization of C. perfringens Isolates for Toxins** H Shivaprasad, *University of California, Davis, Tulare, CA.*	**Evaluation of Efficacy of a Live HVT-vectored IBD Vaccine in a Controlled Study with SPF Chicken against Virulent Challenges with Classic IBDV, vvIBV or IBDV Variant E Type – Clinical Protection, Bursa Health, Viral Shedding** Andreas Herrmann, *Merial SAS, Lyon, France.*
5:15 PM	**Necrotic enteritis: The Struggles Understanding This Syndrome** Steve Fitz-Coy, *Merck Animal Health, Salisbury, MD.*	**A Retrospective Study of IBDV Surveys Comparing Infection Dynamics using Different Broiler Vaccination Strategies** Kalen Cookson, *Zoetis, Lawrenceville, GA.*
5:30 PM	Adjourn	

	Room 221 AB	Room 221 CD
Topic	Blackhead	Bacteria
Moderator	Becky Tilley	Kevin Maschek
8:00 AM	**Future of Blackhead Disease in Poultry** Steven R Clark, *Devenish, West Jefferson, NC.*	**Attempts to Determine the Source of Escherichia coli in Day-old Turkey Poults** Sara Reichelt, *NCSU-CVM Student and Prestage Farms, Inc., Raleigh, NC.*
8:15 AM	**Histomoniasis in Turkey Flocks Before and After the Ban of Nitarsone** Rüdiger Hauck, *University of California, Davis, CA.*	**Avian Pathogenic E. coli in Hens and Day-of Hatch Broilers** Daniel Karunakaran, *Agro BioSciences, Cary, NC.*
8:30 AM	**A Recurrence of Blackhead in Young Turkey Breeder Toms in a House that had Experienced Blackhead Many Years Previously** Eric Gonder, *Butterball LLC, Goldsboro, NC.*	**Bacterial Respiratory Diseases of Turkeys: Diagnosis and Control** Hafez Mohamed Hafez, *Insitute of Poultry Diseases, Free University Berlin, Germany.*
8:45 AM	**Experiences Treating Histomoniasis in Turkeys** Brian Wooming, *Cargill Turkey & Cooked Meats, Springdale, AR.*	**Genotypic and Phenotypic Assessment of Gallibacterium anatis Isolated from Poultry in the United States** Roxana Sanchez Ingunza, *CEVA Animal Health , Lenexa, KS.*
9:00 AM	**Field Experiences Controlling Histomoniasis- Blackhead Disease in Turkeys at a Large Multiage Commercial Growout Facility** Arun K. Bahl, *Bahl Farms Inc/Consulting, Cornelius, NC.*	**Fatty Liver Disease and Osteoporosis (Cage Layer Fatigue): Predisposing Factors or Consequence of Salmonella Gallinarum in Commercial Laying Hens?** Martha Pulido-Landinez, *Mississippi State University, Pearl, MS.*
9:15 AM	**Effects of Feed Additives on the Progression of Blackhead Disease in Turkeys** Robert Beckstead, *The University of Georgia, Athens GA.*	**Prevalence and Antimicrobial Resistance of Campylobacter Species Isolated from Backyard Chickens in Grenada, West Indies** Keshaw Tiwari, *St. George's University, St. Georges, Grenada.*
9:30 AM	**Break**	
Topic	Blackhead (continued)	Bacteria (continued)
Moderator	Kabel Robbins	Kristen Roza
9:45 AM	**Geospatial Mapping of Histomoniasis Outbreaks in Commercial Poultry in the Southeast United States** Rebecca Jones, *North Carolina State University, Raleigh, NC.*	**Microbial Analysis of Bioaerosols in Poultry Houses: A Comparison of Different Poultry Production Types** Mattias Delpont, *Université de Toulouse, INP, ENVT and INRA, Toulouse, France.*
10:00AM	**Blackhead in Pullets- Clinical and Economical Impacts** Deirdre Johnson, *Mountaire Farms, Hebron, MD.*	**A Two-Year Retrospective Study of Bacterial Enumerations from Commercial Broiler Breeder Fecal Samples** John Schleifer, *QTI, Inc., Gillsville, GA.*
10:15AM	**Investigating the Prevalence of Histomonas meleagridis Shedding by Captive Raised Ring-Necked Pheasants (Phasianus colchicus) in Pennsylvania** Richard Gerhold, *University of Tennessee, Knoxville, TN.*	**Cross-Protective Bacterins for E. coli and Salmonella** Margie Lee, *UGA, Watkinsville, GA.*
Topic	Blackhead (continued)	Antibiotics
10:30AM	**Overview of Field and Experimental Experiences with Paromomycin as Treatment for Histomonosis in Turkeys** Koen De Gussem, *Huvepharma, NV, Belgium.*	**Quantifying Antimicrobial use in Poultry Production** Randall Singer, *University of Minnesota, Saint Paul, MN.*
10:45AM	**A Clonal Culture as Key Tool to Develop New Diagnostics and a Vaccine against Histomonosis** Michael Hess, *University of Veterinary Medicine Vienna, Austria*	**Antimicrobial Resistance: Clues Policy Changes Won't Affect the Outcome.** Hector Cervantes, *Phibro Animal Health, Watkinsville, GA.*
11:00AM	**Break**	

Monday, August 8, 2016 (continued)

Moderator	Bruce Calnek	
11:15 AM	Lasher-Eckroade History Lecture: **The History of the First Generation Marek's Disease Vaccines: The Science and Little Known Facts** Karel Schat, Cornell University, Ithaca, NY. **Rooms 221AB**	
12:00 PM	**AAAP Awards Luncheon** **Marriot River Center Hotel Room: Salon E**	
	Room 221 AB	**Room 221 CD**
Topic	Coccidiosis	Food Safety
Moderator	Charles Broussard	Denise Brinson
2:00 PM	**Coccidiosis Vaccination Revealed: A Peek Behind the Curtain** G. Donald Ritter, *Mountaire Farms Inc., Salisbury, MD.*	**Examination of the Environmental Reservoir of Resistance Genes for Foodborne Pathogens in Poultry Production.** Karen Liljebjelke, *University of Calgary, Calgary, Canada.*
2:15 PM	**Comparison of Breeder/Layer Coccidiosis Vaccines** Linnea Newman, *Merck Animal Health, North Creek, NY.*	**Are Salmonella from Poultry Distinct from Those Present in the Environment?** John Maurer, *University of Georgia, Athens, GA.*
2:30PM	**Evaluation of Gel Application of a Coccidia Vaccine** Grace Ashby, *University of Georgia, Athens, GA.*	**Colonization of Internal Organs by Salmonella Enteritidis in Experimentally Infected Laying Hens Housed in Enriched Colony Cages at Different Stocking Densities** Richard Gast, *U.S.National Poultry Research Center, USDA-ARS, Athens, GA.*
2:45 PM	**Application of a Coccidia Vaccine and the Evaluation of Field Scoring Methods** Seiche Genger, *North Carolina State University, Raleigh, NC.*	**The Impact on Intestinal Colonization with Salmonella enterica serovar Typhimurium with Partial Replacement of NaHCO3 in Diet** Dulmelis Sandu, *University of Georgia, PDRC, Athens, GA.*
3:00 PM	**Rate of Ingestion/Uptake of Eimeria oocysts from Live Coccidia Vaccines via Different Application Methods of Inoculation** Sue Hubbard, *Merck Animal Health, Mt. Olive, MS.*	**Using Bioluminescent Salmonella to Identify Infection Sites that Might Contribute to Contamination of Ground Chicken Meat** Monique Franca, *UGA, Athens, GA.*
3:15 PM	**Break**	
Topic	Coccidiosis (continued)	Food Safety (contiued)
Moderator	Sara Steinlage	Elena Behnke
3:30PM	**Comparative Evaluation of Different Anticoccidial programs in Broilers with Specific Attention to Avatec 20 (Lasalocid Sodium)** Babak Sanei, *Zoetis Canada Inc., Canada*	**The Importance of Data in Salmonella Risk Mitigation: Development of a Cloud-based Technical Platform for Food Safety Management in Poultry Production** Robert O'Connor, *Foster Farms, Livingston, CA.*
3:45 PM	**Dynamics of Eimeria Oocyst Concentrations and Species Composition in Commercial Broiler Houses During Anticoccidial Drug or Vaccine Programs** Mark Jenkins, *ARS-USDA, Beltsville, MD.*	**Utilization of Next Generation Sequencing to Evaluate Vaccination against Salmonella Typhimurium in an US Broiler Integrator** John ElAttrache, *CEVA Animal Health, Lenexa, KS.*
Topic	Coccidiosis (continued)	Welfare
4:00 PM	**Evaluation of the Efficacy of Different Coccidiosis Bioshuttle Programs and Dietary Capsicum-turmeric Oleoresins on Broiler Performance** Yun-Ting Wang, *Huvepharma, Dallas, TX.*	**Application of Six Sigma Principles and Methodology to Improve Animal Welfare: Reduction in Wing Damage** Kenneth Opengart, *Keystone Foods, Signal Mountain, TN.*
4:15 PM	**What are Some Potential Alternatives to AGPs and Ionophores in an Antibiotic Free Program?** Tina Yun-Ting Wang, *Huvepharma, Atlanta, GA*	**Development and Implementation of a Progressive Welfare Index (PWI) as a Real-time Indicator of Overall Welfare Performance** Kenneth Opengart, *Keystone Foods, Signal Mountain, TN.*
4:30 PM	**Wine & Cheese Social** Poster Room 220	
5:30 PM	Adjourn	

	Room 221 AB	Room 221 CD
Topic	Marek's Disease Virus	Diagnostics
Moderator	Pedro Villegas	Kalen Cookson
8:00 AM	**Effect of Probiotics on Marek's Disease Vaccination** John Dunn, *USDA-ARS-ADOL, East Lansing, MI.*	**Database Mining of Normal Water Biochemical Paramaters of Water in Poultry Barns in the Province of Québec and the Effect of Different Water Additives and Antibiotics on these Parameters.** Jean Pierre Vaillancourt, *University of Montreal, St. Hyacinthe, Canada.*
8:15 AM	**Rimler Award Winner** * **Evaluation of Viral and Host Mechanisms Involved in Permanent Marek'S Disease Virus Induced Immunosuppression** Faiz Nik Mohd Azmi, *North Carolina State University, Raleigh, NC.*	**Investigating Respiratory Physiology and Thermoregulation in Poultry using Non-Invasive Point-of-Care Methods** Marie Souvestre, *Université de Toulouse, Toulouse, France.*
8:30 AM	**In Ovo Vaccination of Commercial Meat Type Chickens with Herpesvirus of Turkey: Vaccine Replication and Effect on the Chicken Immune System** Isabel Gimeno, *North Carolina State University, Raleigh, NC.*	**Monitoring Cleaning and Disinfection Protocols with the use of AccuPoint as Hygiene Screening Tool in Hatcheries** Ricardo Munoz, *Neogen, Lexington, KY.*
8:45 AM	**Assessment of the Signaling Synergism of Bivalent HVT/SB1 Vaccine Components on Innate Sensing and Acquired Immune Patterning** Sabarinath Neerukonda, *University of Delaware, Newark, DE.*	**Pathology of Wooden Breast Disease in Modern Broiler Chickens: A Histologic and Ultrastructural study** Michael Babak, *University of Delaware, Newark, DE.*
Topic	Pox	Enteric Health
9:00 AM	**Co-infection with Fowlpox Virus Strains Carrying Variable Sequences of Reticuloendotheliosis Virus** Deoki Tripathy, *University of Illinois, Urbana, IL.*	**HTS: A Unique, Global Surveillance System for Monitoring and Benchmarking Enteric Health** Alexandre Zocche, *Elanco Animal Health, Greenfield, IN.*
9:15 AM	**Nucleotide Sequence Analysis of Selected Genes of Avianpox Viruses** Deoki Tripathy, *University of Illinois, Urbana, IL.*	**Intestinal Parasitic Worms are a Common Problem in the Poultry Industry.** Blayne Mozisek, *Merck Animal Health, Austin, TX.*
9:30 AM	**Outbreaks of Acute Respiratory Fowlpox in Layers in France** Jean-Luc Guerin, *Université de Toulouse, INP, ENVT and INRA, Toulouse, France*	**Comparison of Specific Intestinal Wet Mount and Histological Findings** Stephen Collett, *University of Georgia, Athens, GA.*
9:45 AM	**Break**	
Topic	Virology/Newcastle Disease Virus	Enteric Health (continued)
Moderator	Armando Mirande	Nathaniel Tablante
10:00 AM	**Application of Next-Generation Sequencing (NGS) in Diagnostic Avian Virology** Huaguang Lu, *The Pennsylvania State University, University Park, PA.*	**Development of Real-Time PCR Reagents to Identify Salmonella DNA in Enriched Cultures** Kristin Mesires, *IDEXX Laboratories Inc., Westbrook, ME.*
10:15 AM	**Time as Critical Parameter for Vaccination of Turkeys against Newcastle Disease** Christian Grund, *FLI, Federal Research Institute for Animal Health, Greifswald, Germany.*	**Immunohistochemical Characterization of Jejunal Epithelial Cell Populations in Young Turkeys with Depressed Growth** Rebecca Jones, *North Carolina State University, Raleigh, NC.*
10:30 AM	**Evaluation of in Vivo Replication of a Newcastle Disease Live Attenuated Vaccine Strain in Commercial Broiler Chicks: A Comparison of Drinking-Water, Spray and Eye Drop Vaccination Methods** Andrea Delvecchio, *MERIAL SAS, Lyon, France.*	**Review of Intestinal Wet Mount Examination** Stephen Collett, *University of Georgia, Athens, GA.*
Topic	Newcastle Disease Virus	Clostridia
10:45 AM	**Effect of Live or Live & Inactivated Newcastle Vaccination Programs against Virulent Newcastle Disease Virus Genotype VII Challenge in Controlled Studies with SPF Chicken or Commercial Layer – Clinical Protection, Viral Shedding** Andreas Herrmann, *Merial SAS, Lyon, France.*	**Gangrenous Dermatitis in Turkeys Associated with Used Litter in Extended Dormancy** Sam Hendrix, *Colorado Quality Research, Wellington, CO.*
11:00 AM	**Productivity, Clinical Performance and Seroconversion of Broilers Vaccinated with a Vector rHVT-F Protein Newcastle Disease (ND) Vaccine and Reared in Non-Vaccinating and Velogenic ND Virus (vNDV)- Free Poultry Companies.** Luiz Sesti, *CEVA Animal Health, Rio Claro, Brazil.*	**Comparison of the Proteins Produced by Severe Necrotic Enteritis (NE) Producing Strains of C. perfringens (netB positive) to those of a NetB Positive non NE Producing Strain** Joan Smyth, *University of Connecticut, Storrs, CT.*

Tuesday, August 9, 2016 (continued)

Topic	Newcastle Disease Virus (continued)	Clostridia (continued)
Moderator	Armando Mirande	Nathaniel Tablante
	Room 221 AB	**Room 221 CD**
11:15 AM	**Repeated Challenge does not Decrease the Efficacy of NDV Vaccines** Patti Miller, *Southeast Poultry Research Laboratory, Athens, GA.*	**Feeding Tributyrin or Organic Acid Blend with Essential Oils Administered in the Drinking Water to Broilers to Reduce Clostridium perfringens Induced Necrotic Enteritis** Greg Mathis, *Southern Poultry Research, Inc., Athens, GA.*
11:30 AM	**Characterization of NDV Field Isolates and Evaluation of Protection against an NDV Isolate by a Recombinant HVT-ND and a Live Attenuated (C2) Vaccines in Commercial Broilers** Ivan Alvarado, *Merck Animal Health, Athens, GA.*	**Impact of Controlling Bacteria in Feed on Broiler Performance during a Clostridial Challenge** Kurt Richardson, *Anitox Corp, Lawrenceville, GA.*
11:45 AM	**The use of Next Generation Sequencing in the Diagnosis and Typing of NDV Isolates from Commercial Poultry Flocks** Salman Latif Butt, *University of Georgia, Athens, GA.*	**2015 Bacterial Enteritis Global Impact Assessment** Alexandre Zocche, *Elanco Animal Health, Fishers, IN.*
12:00 PM	**Lunch**	

Topic	Wealth of Knowledge	Mycoplasma
Moderator	Brian Jordan	Vijay Durairaj
1:15 PM	**National Poultry Improvement Plan** Denise Brinson, *USDA-APHIS, Conyers, GA.*	**Reed Rumsey Award Winner** * **Study on the Prevalence and Association of a Novel Mycoplasma sp. with Reproductive Disease in Commercial Goose Breeders.** Silvia Carnaccini, *CAHFS-UCDavis, Turlock, CA.*
1:30 PM	**The Commercial Duck Industry in the United States. Current Disease Challenges and Contributions to the US Poultry Industry** Jaime Ruiz, *Elanco Animal Health, McKinney, TX.*	**Mycoplasma synoviae Antimicrobial Susceptibility Testing** Naola Ferguson-Noel, *University of Georgia, Athens, GA.*
1:45 PM	**Tools for the Poultry Professional: How to Increase Personal and Professional Wellness** Andrea Zedek, *Zedek Poultry Consulting, LLC, Simpsonville, SC.*	**Evaluation of Infectious Laryngotracheitis CEO Vaccine in Mycoplasma synoviae Positive Broilers** Victoria Drouet, *University of Georgia, PDRC, Athens, GA.*
Topic	Infectious Bronchitis Virus	Laryngotracheitis
2:00 PM	**Emerging IBV Variants: The Genotypes, Serotypes and Pathotypes** Hui-Wen Chen, *National Taiwan University, Taipei, Taiwan.*	**Cytokine expression patterns in conjunctiva, Harderian gland and trachea after ocular or oral inoculation with a virulent strain of infectious laryngotracheitis virus (ILTV)** Gabriela Beltrán, *University of Georgia, Athens, GA.*
2:15 PM	**Use of Infectious Bronchitis Serotype Specific Probes for Egg Layers and Broiler Breeders** Louise Dufour-Zavala, *GPLN, Gainesville, GA.*	**Serologic Monitoring (Vaccine Take) after Innovax ILT (rHVT-ILT) Vaccination using Conventional Type of ILT Elisa Kits and an ILT gI Specific Protein Elisa Kit.** Rik Koopman, *MSD AH, Boxmeer, Netherlands.*
Topic	Infectious Bronchitis Virus (continued)	Reovirus
2:30 PM	**Beyond Diagnosis: Molecular Epidemiological Analysis of Infectious Bronchitis Virus** Mark Jackwood, *UGA, Athens, GA.*	**Pathogenicity of Two Variant Reovirus Isolates from Clinical Cases of Viral Arthritis in Arkansas and North Carolina** Tyler Gamble, *The University of Georgia, PDRC, Athens, GA.*
2:45 PM	**Molecular Characterization of Infectious Bronchitis Viruses from Broiler Chicken Farms in Peru** Eliana Icochea, *University of San Marcos, Lima, Peru.*	**Turkey Arthritis Reovirus; Pathogenesis and Immune Response** Tamer Sharafeldin, *University of Minnesota, Saint Paul, MN.*

3:00 PM	Break	
	Room 221 AB	**Room 221CD**
Topic	Infectious Bronchitis Virus (continued)	Management
Moderator	Andres Montoya	Susan Trock
3:15 PM	**Role of Spike S2 Ectodomain in Attachment and Selection of ArkDPI IBV Vaccine Subpopulations in Chickens** Vicky van Santen, *Auburn University, Auburn, AL.*	**Chick Quality and First Week Field Evaluations and their Relationship to Incubation and Brooding Conditions** Donna Hill, *Donna Hill Consulting, Mountain Home, AR.*
3:30 PM	**Characterization of Kidney Cell-Adapted IBV ArkDPI Vaccine during Back-Passages in Embryonated Eggs** Haroldo Toro, *Auburn Unversity, Auburn, AL.*	**Effects of an Automated Egg Sanitizing Machine using both Hydrogen Peroxide with UV Light to Reduce Bacterial Contamination and Improve Hatchability in Older Hen Flocks** Myles Hill, *Elanco Animal Health, Bentonville, AR.*
3:45 PM	**Evaluation of the Changes in IBV Subpopulation Composition using Next Generation Sequencing** Ha-Jung Roh, *CEVA biomune, Leawood, KS.*	**Incubation Temperature and its Effect on Chick Development and Avian Pathology** Danuta Furmanek, *Poultry Performance Plus, Niemcz, Poland.*
4:00 PM	**Major Histocompatibility Complex and Innate Immunity as Factors Providing Genetic Resistance to Infectious Bronchitis Virus** Rodrigo Gallardo, *University of California, Davis, Davis, CA.*	**The Georgia Avian Influenza Hotline- Design, Creation, and Implementation** Len Chappell, *The Georgia Avian Influenza (AI) Hotline, Gainesville, GA.*
4:15 PM	**Is Infectious Bronchitis Vaccination with Mass and QX Genotypes Protective Against Q1 Strain?** Corrado Longoni, *Merck Animal Health, Milano, Italy.*	**Use of a Highly Pathogenic Avian Influenza (HPAI) Hot Line to Improve Early Detection of Possible HPAI Infection in Small (Backyard) Flocks in Georgia.** Douglas Anderson, *GPLN, Forsyth, GA.*
4:30 PM	**Control of Severe Respiratory Problems Caused by Arkansas-Related Strains of the Infectious Bronchitis Virus Using a Combination of Two Heterologous IB Vaccine Strains in Broilers In Mexico** Francisco Rios-Cambre, *BSc., Santiago Tianguistenco, Mexico.*	**Evaluation of Toe-Trimming Strategies in Commercial Turkey Hens** Michael Martin, *North Carolina State University, CVM, Morrisville, NC.*
4:45 PM	**Evaluating Infectious Bronchitis Virus Vaccination by Gel Administration** Brian Jordan, *The University of Georgia, Athens, GA.*	**Mortality Surveys, An Important Tool to Evaluate Broiler Breeder Health** Jose Bruzual, *Aviagen, Inc., Dacula, GA.*
5:00 PM	Adjourn	

AAAP Poster Session

Room 220

Antibiotic Resistance/Susceptibility

Antibiotic Susceptibility of Escherichia coli Isolated from Vertebral Osteomyelitis of Broilers in Brazil Roselene Ecco**,** *Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. (#1)*

Impact of Ceftiofur Withdrawal from a Canadian Hatchery on Cephalosporin Resistance in Extra-Intestinal Pathogenic Escherichia coli Luc Verrette, *Université de Montréal, St-Hyacinthe, Canada. (#3)*

Mobility of Antimicrobial Resistance in Avian Pathogenic Escherichia coli (APEC). Catherine Logue, *Iowa State University, Ames, IA. (#4)*

Trends in Antibiotic Resistance to Salmonella Enteritidis Phage Types Isolated from Poultry from 2007 to 2015 Thomas Denagamage, *The Pennsylvania State University, University Park, PA. (#6)*

Avian Influenza

A Preliminary Survey of Hemagglutinating Viruses from Ducks in the Province of Buenos Aires, Argentina. Celina Buscaglia, *Comision de Investigaciones Cientificas de la Provincia de Buenos Aires, MB Gonnet, Argentina. (#7)*

A Synthetic Vaccine of Biodegradable Microspheres for Controlled Release of Adjuvant and Femtomole-Dosed Peptide Antigens Protects against Infectious Bursal Disease Virus. Joseph Giambrone, *Auburn University, Auburn, AL. (#8)*

Cost-Effectiveness of Avian Influenza Vaccination Strategies In Bangladesh: Added Value to Day Old Chick Vaccination Fernando Lozano, *CIRAD- AGIRs, Libourne, France. (#10)*

Litter Composting Procedures for Inactivation of Avian Influenza Virus in Eggs Teresa Dormitorio, *Auburn University, Auburn, AL. (#11)*

Network Modeling of Poultry Production and Immunity Levels: Analysis and Perspectives for Vaccination Strategy and Control of Highly Pathogenic Avian Influenza in Vietnam Fernando Lozano, *CEVA Animal Health, Libourne, France. (#12)*

Surveillance of Amino Acid Substitutions in Avian Influenza Viruses Isolated from Wild Birds from South Korea, 2010-2015 Kwang-Hyun Oh, *Chungbuk National University, CVM, Cheongju, South Korea. (#13)*

The Pathobiology of Highly Pathogenic H5N2 Avian Influenza Virus in Ruddy Ducks and Lesser Scaup Erica Spackman, *SEPRL-USDA-ARS, Athens, GA. (#14)*

Understanding New Highly Pathogenic Avian Influenza (AI) Viruses Affecting the U.S. Poultry Industry and their Persistence Rodrigo Gallardo, *University of California, Davis, Davis, CA. (#15)*

Biosecurity

Biosecurity Risk Survey for Grandparent farms in Latin America Jose Bruzual, *Aviagen, Inc., Dacula, GA. (#16)*

Blackhead

Clinical and Pathological Characteristics of a Histomoniasis (Blackhead) in Backyard Poultry in Vietnam Jong-Suk Mo, *Chungbuk National University, CVM, Cheongju, South Korea. (#17)*

Increased Concerns for the Lack of Therapeutic Interventions against Histomoniasis (Blackhead Disease) in Turkeys Prajwal R. Regmi, *U.S. Food and Drug Administration, Rockville, MD. (#18)*

Regulatory Considerations for Approval of Drugs against Histomoniasis in Turkeys and Gamebirds Prajwal R. Regmi, *U.S. Food and Drug Administration, Rockville, MD. (#19)*

The Efficacy of Three Hydroxyquinolones Against Histomonas meleagridis Growth in an In-Vitro Assay. Lorraine Fuller, *University of Georgia, PDRC, Athens, GA. (#20)*

Case Reports

An Outbreak of Adenoviral Tracheitis in Commercial Goslings in France Mattias Delpont, *Arzacq-Arraziguet, France. (#21)*

Chondronecrosis with Osteomyelitis Caused by Enterococcus cecorum in Broiler, Korea You-Chan Bae, *Avian Disease Division, Animal and Plant Quarantine Agency, Anyang, Republic of Korea. (#22)*

Fowl Tick (Argas persicus) Infestation in a Backyard Chicken Flock Jarra Jagne, *Cornell University, Ithaca, NY. (#23)*

HPAI H5N8 Outbreak in Commercial Pekin Ducks in California H Shivaprasad, *University of California, Davis, Tulare, CA. (#25)*

Tetratrichomoniasis-associated Mortality in Four Ducks and One Pheasant at an Avian Conservation Park in Louisiana Nobuko Wakamatsu, *Louisiana State University, SVM, DPS, Baton Rouge, LA. (#26)*

The Fowl Pox Cases with Co-infection of Infectious Laryngotracheitis Virus in Chicken Van Dam Lai, *Chungbuk National University, CVM, Cheongju, South Korea. (#27)*

Diagnostics

A Universal One-Step Real-Time RT-PCR for Detection of all Avian Orthoreovirus Genotypes Lin Lin, *Penn State University, University Park, PA. (#28)*

Correlation between the 1 Day Chick Quality Determined by the Cervantes Test with the First-Week Mortality in the Broilers Farms in the Period September 2014 - February 2015 Laura Milano, *Diagnostic Laboratory, Protinal - Proagro, Valencia, Venezuela. (#29)*

Effect of Water Electrolyte Balance or Strong Ion Difference (SID) on Acid-base Balance of Broiler Chickens Daniel Venne, *Couvoir Scott ltée, St-Lambert de Lauzon, Canada. (#30)*

Frequency of Swollen Head Syndrome in Broilers from Peru Nelly Cribillero, *San Marcos University, Lima, Peru. (#31)*

Gross and Histologic Diagnosis of Retrograde Yolk Inhalation in Poultry John Roberts, *Thompson Bishop Sparks State Lab, Auburn, AL. (#32)*

Specificity Study of ELISA Kits Brenda Glidewell, *GA Poultry Lab, Gainesville, GA. (#33)*

The Use of Vaccinated Sentinel Chickens for Isolation of Anitgenic Variants of Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease Virus (IBDV) from Delmarva Broilers John Rosenberger, *AviServe LLC, Newark, DE. (#34)*

Enteric Health

A Comparison of Fungal and Bacterial Populations in Broilers from High and Low Producing Farms. J. Allen Byrd, *USDA, ARS, College Station, TX. (#35)*

Chicken Astrovirus is Associated with Enteric Disease in Experimentally Infected Chickens Luis Nuñez, *FMVZ-USP, Sao Paulo, Brazil. (#36)*

Effect of Dietary Vitamin E on Eimeria tenella-induced Oxidative Stress in Broiler Chickens Rezvan Kiani, *Mehr Specialized Poultry Center, Amol, Iran. (#37)*

Gut Health in Poultry 2016 Maarten De Gussem, *Vetworks, Aalter, Belgium (#38)*

Non-Salmonella Associated Cecal Cores In Turkey Poults Dave Fernandez, *AgForte (#39)*

Food Safety

Characterizing Outer Membrane Proteins of Salmonella Enteritidis Expressed in Egg yolk Dona Saumya Wijetunge, *Pennsylvania State University, State College, PA. (#41)*

Effectiveness Assessment of a Genetically Modified Live Vaccine in Broilers Challenged with Salmonella Heidelberg Eduardo Muniz, *Zoetis, Jaragua do Sul, Brazil. (#42)*

Evaluation of the Effectiveness of Various Doses of Bacillus subtilis Probiotic with or without Mannan in Prevention of Salmonella Heidelberg Colonization. Charles Hofacre, *University of Georgia, Athens, GA. (#43)*

Identification of Extended-Spectrum Beta-Lactamases (ESBL) in E. coli Strains Isolated from Chicken Carcasses Obtained from Processing Plant, Public Markets and Supermarkets in Mexico Patrick Dominguez, *Bachoco SA de CV, Mexico City, Mexico. (#44)*

Microbiological Profile of Carcasses in Differents Points of the Production Line of a Birds Processing Plant Viamney Yanes, *Diagnostic Laboratory, Protinal - Proagro, Valencia, Venezuela. (#45)*

MLST Genotype of Campylobacter spp. Isolated from Indigenous Chickens in Grenada, West Indies Ravindra Sharma, *St George's University, St. George, Grenada. (#46)*

Molecular Epidemiology of Salmonella Heidelberg Isolated from Chickens and Turkeys Kakambi Nagaraja, *University of Minnesota, Saint Paul, MN. (#47)*

Salmonella Enteritidis in Shell Eggs Produced by Backyard and Other Small Layer Flocks Subhashinie Kariyawasam, *Pennsylvania State University, University Park, PA. (#48)*

The Quebec SE Committee, a Successful Industry - Governmental Agencies - Academia Collaboration Martine Boulianne, *Université de Montréal, St. Hyacinthe, Canada. (#49)*

Immunology

An Educational Tool to get References of Healthy Bursa of Fabricius in Commercial Broilers. Christophe Cazaban, *CEVA Animal Health, Libourne, France. (#50)*

Assessment of *in-ovo* Broiler Substrate Deposition Site at Different Embryo Developmental Stages from Three Breeder Flock Ages using a Differentiated Injection System Fernando Lozano, *CEVA Animal Health, Libourne, France. (#51)*

Evaluation of Gimax® Solution as an Immunostimulant Agent in Broiler Chickens Rezvan Kiani, *Mehr Specialized Poultry Center, Amol, Iran. (#52)*

Infectious Bronchitis Virus

Can Three Strains of Infectious Bronchitis Virus Given Simultaneously Induce Protection in Chickens? Brian Ladman, *University of Delaware, Newark, DE. (#53)*

Comparison of Protection Induced by Three Different Types of 793/B like Commercial Infectious Bronchitis Virus (IBV) Vaccines against Challenge with IS-1494/06 Like IBV Genotype Arash Ghalyanchilangeroudi, *University of Tehran, Tehran, Iran. (#54)*

Efficacy Studies of a Variant Infectious Bronchitis Virus Vaccine Brianna Ford, *CEVA Animal Health, Lawrence, KS (#55)*

Homologous and Heterologous Protection against Brazilian BR-I Viruses of Infectious Bronchitis Jorge Chacón, *CEVA Animal Health, Campinas, Brazil. (#56)*

Infectious Bronchitis Viruses Isolated during 2005-2014 from Broiler Chicken Farms in Peru Rosa Gonzalez, *San Marcos University, Lima, Peru. (#57)*

Insertions in the S1 Spike Glycoprotein of GA13-Type Infectious Bronchitis Virus (IBV) Affect Binding to Chicken Tissues Emily Aston, *University of Georgia, PDRC, Athens, GA. (#58)*

Multi-Strain Infection by Infectious Bronchitis Variant Viruses in Broiler and Breeder Flocks in Latin America Luiz Sesti, *CEVA Animal Health, Rio Claro, Brazil. (#59)*

Pathogen Associated Molecular Pattern (PAMP) Receptor Expression in Chickens Before and After IBV Challenge Isabelle Kallenberg, *Auburn University, Auburn, AL. (#60)*

Pathogenicity Study of a New Emerging Iranian Infectious Bronchitis Virus Variant (IR-1) in Experimentally Infected SPF Chickens Arash Ghalyanchilangeroudi, *University of Tehran, Tehran, Iran. (#61)*

Peptide Nanoparticle-based Vaccine for Infectious Bronchitis Virus Jlanping Li, *University of Connecticut, Storrs, CT. (#62)*

Rapid and Specific Identification of Infectious Bronchitis Virus (IBV) by Real Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) with an Internal Positive Control Jongseo Mo, *University of Georgia, PDRC, Athens, GA. (#63)*

Use of 9CFR Part 107.1 (b) to Control a Variant Strain of Nephropathogenic Infectious Bronchitis Virus in Delmarva Broiler Chickens Jack Gelb, Jr., *University of Delaware, Newark, DE. (#64)*

Assessment of an Immune-Complex Infectious Bursal Disease Vaccine in "Low" Maternally-Immune Commercial Broiler Chicks. Christophe Cazaban, *CEVA Animal Health, Libourne, France (#65)*

Chicken Melanoma Differentiation-Associated Gene 5-Dependent Innate Immunity Bridging Adaptive Immunity in Infectious Bursal Disease Virus Infection Tsang Long Lin, *Purdue University, CVM, West Lafayette, IN. (#66)*

Molecular Epidemiologic Study of Infectious Bursal Disease Viruses in Brazilian Poultry Farms under Different Vaccination Programs Eduardo Muniz, *Zoetis, Jaragua do Sul, Brazil (#67)*

Laryngotracheitis

Quantitation of Infectious Laryngotracheitis Virus by a Combination of Virus Propagation in Cell Culture and Quantitative Real-Time PCR Girish Sarma, *Hygieia Bio. Lab, Woodland, CA. (#70)*

Management

The Effects of Setting Hatching Eggs Upside Down on Embryonic Development and Hatchability Myles Hill, *Elanco Animal Health, Bentonville, AR. (#71)*

Marek's Disease Virus

Characterization of BACrMd5-REV-LTR Virus as MD Vaccine in Commercial Meat Type Chickens: Protection and Immunosuppression Aneg Lucia Cortes, *North Carolina State University, CVM, Raleigh, NC. (#72)*

Effect of Marek's Disease Vaccines on the Immune Responses on Chicken Embryos Ayanna Glaize, *North Carolina State University, Raeford, NC. (#73)*

Induction of Unfolded Protein Response (UPR) by Marek's Disease Virus (MDV). Sabarinath Neerukonda, *University of Delaware, Newark, DE. (#74)*

Pathogenesis of Marek's Disease Vaccine in Turkey Embryos William Shaw, *North Carolina State University, Raleigh, NC. (#75)*

Mycoplasma

Current Status of Mycoplasma gallisepticum after Starting an Eradication Plan in a Grandparent Breeder Flock in Venezuela. Dayana Perez, *Protinal Proagro, Valencia, Venezuela. (#76)*

Increasing Virulence of a Mycoplasma synoviae Outbreak Strain from Northeast Georgia Amanda Olivier, *University of Georgia, Athens, GA. (#77)*

Newcastle Disease Virus

Effect of Field Conditions on the Serological Response to Vaccination against Newcastle Disease Virus. Francisco Perozo, *University of Zulia, Venezuela (#78)*

Vaccine/Challenge Assessment of Current Newcastle Disease Vaccination Protocols in Endemic Countries. Rosmar Marcano Martí, *University Central of Venezuela (#79)*

Virology: Other

Onsite Surgical Procedures for Backyard Poultry Annika McKillop, *UMcKillop Poultry Medicine, Frederick, MD. (#80)*

Detection and Characterization of Two Co-infection Variant Strains of Avian Orthoreovirus (ARV) in Young Layer Chickens using Next-Generation Sequencing (NGS) Yi Tang, *The Pennsylvania State University, University Park, PA. (#81)*

Evaluation of the Safety and Efficacy of a Chicken Embryo Origin Fowl Pox Virus Vaccine in One-Day-Old Broiler Chicks Girish Sarma, *Hygieia Bio. Lab, Woodland, CA. (#82)*

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Welfare

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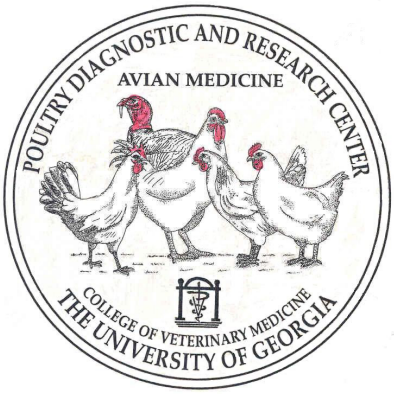

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AAAP/AVMA Future Annual Convention dates and locations

Future Convention Cities

2017	July 21-25	Indianapolis, IN
2018	July 13-17	Denver, CO
2019	July 19-23	Washington, D.C.
2020	July 30-August 4	San Diego, CA

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The American Association of Avian Pathologists promotes scientific knowledge to enhance the health, well-being, and productivity of poultry to provide safe and abundant food for the world.

Proceedings of the 2016 AAAP Annual Meeting

Abstracts listed by order of presentation

Symposium

Consumer Perspectives on the Safety of Poultry Products

Robert O'Connor

Foster Farms

Poultry has been linked to foodborne illnesses caused by Salmonella spp. and Campylobacter spp. A 2014 University of California research project, funded in part by Foster Farms, reported on observed handling behavior when 120 volunteers prepared chicken and salad in their homes. Post-meal preparation/video recording, each volunteer completed a questionnaire related to their food safety attitudes and knowledge. The consumer responses to the questionnaire demonstrated that they had perceived knowledge of safe-food handling, and had personally heard of people becoming ill from eating poultry products. In practice though, as revealed by the video recording, another set of results emerged regarding consumer behavior. This study confirms that measures can be taken to mitigate the risk of food safety illnesses caused by the handling and cooking of raw poultry products. The industry should not abdicate the responsibility for educating consumers buying raw poultry to government agencies, consumer activist groups, and public or in-home education. Evoking the "one medicine" approach, the poultry industry must accept its role in making its final product safer, not just through interventions in the field or processing plant, but also in their customer's kitchen. This approach, industry shouldering some direct responsibility for the safety of the end-user's experience and messaging appropriately, is not novel. Data demonstrates this to be the case with other consumer packaged goods; alcoholic beverages being a primary example.

Practical Methods of Avian Influenza Prevention and Control to Protect Poultry and Humans

Dr. Jack Shere, Dr. Deborah Nelson

Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture

Biosecurity approaches fall into two categories. Structural biosecurity is built into the physical construction and maintenance of a facility. Operational biosecurity encompasses the standard operating procedures (SOPs) that minimize the chance of virus entering the poultry house and compliance with those SOPs. Over the long term, poultry producers will need to consider both operational and structural biosecurity to reduce their overall risk of HPAI.

Based on expert opinion and experience in the recent outbreak, the highest risks for HPAI virus introduction are personnel who enter the poultry buildings, shared equipment and shared crews, procedures for disposal of dead birds, and manure management. These elements should be the highest priority in allocating resources for improved biosecurity. Further, three concepts may be new to most existing biosecurity plans and should be strongly considered for all commercial operations: a biosecurity officer, a line of separation for each building, and a perimeter buffer area.

Effective biosecurity requires vigilance; producers should put a system in place to verify that biosecurity enhancements are being followed. While there were no human cases of influenza associated with the HPAI outbreaks in 2014-2016, some strains of avian influenza are known to be zoonotic. Rare human infections with some avian viruses have occurred, most often after unprotected

contact with infected birds or contaminated surfaces. Responders must take precautions to minimize their contact with virus-contaminated material, including safe work practices, and proper fitting and use of personal protective equipment. Seasonal influenza vaccination is encouraged.

The Interface Between Public Health and Commercial Poultry Production: A View of Things to Come

F. William Pierson, MS, DVM, PhD, DACPV

Center for Molecular Medicine and Infectious Diseases, Department of Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech

Public health as it pertains to commercial poultry production, whether it is related to food safety, food defense, occupational safety/health or environmental concerns, is an evolving landscape. Pressure from consumers, their representatives, and government agencies tend to drive industry change. Addressing the moral imperative before it becomes a legal one is always good business (GM is compelling example) and a more proactive rather than reactive approach can positively influence public perception and reduce the need for regulation. Issues that may be perceived or truly become threats to public health can potentially be predicted if we look at historical patterns. Topics such as: 1) agroterrorism, 2) the evolution of *Salmonella* serovars, 3) ESKAPE (MDR strains), CREs and *Listeria* (as food/workplace pathogens), 4) antibiotics, inorganic arsenic and disinfectants as environmental toxicants / down-stream food-chain residues, and 5) acquired hypersensitivities to airborne particulates of poultry origin will be discussed. Past trends, current findings, and plausible extrapolations will be explored with the intent of promoting science-based, pre-emptive action to protect consumers, employees and the public trust.

Recent Avian Influenza Outbreaks in Poultry and Humans: An Epidemiologic Perspective

David E. Swayne

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

High pathogenicity avian influenza (AI) viruses (HPAIV) has caused 37 epizootics in poultry and wild birds since 1959. Low pathogenicity avian influenza viruses (LPAIV) are ubiquitous in many migratory waterfowl and shorebirds. H9N2 LPAIV is endemic in poultry populations of North Africa and Asia and has caused tremendous negative economic impact on poultry production. The majority of the HPAIV epizootics have been small with rapid elimination by stamping-out programs. The largest epizootic, H5Nx goose Guangdong (Gs/GD) lineage (i.e. panzootic), has killed or resulted in culling of over 500 million poultry and has affected 70 countries. This single outbreak is larger than the other 36 epizootics combined. The mode of farm-to-farm spread has varied, but movement of fomites through human activity is the main method with minor contribution by air dispersion. LPAIV and HPAIV have caused sporadic human infections mainly by the H5N1 Gs/GD lineage HPAIV (1996-present), H7N7 HPAIV in the Netherlands (2003), H7N9 LPAIV in China (2013-present), and rarely with other HPAIV (e.g. H7N3) and LPAIV (e.g. H9N2). Approximately 1200 human AIV infections have been identified in the past 20 years and with a near 60% fatality rate. By comparison, human seasonal influenza infect billions of people and 300,000-500,000 die annually. The human AIV infections resulted from direct

exposure to infected live poultry with highest risk of infection for farm workers (e.g. H7N7) and consumers in contact with live poultry markets (H5N1 and H7N9). Consumption of infected poultry products has not been a risk factor and has not been a food safety issue.

How epidemiology is used in investigating poultry disease outbreaks

Jean-Pierre Vaillancourt

Department of Clinical Sciences, Faculty of Veterinary Medicine
University of Montreal

When a disease outbreak is first reported, many key questions arise relative to the origin of the problem and any associated risk factors or determinants of the disease. In poultry, epidemiological methods are commonly applied to the investigation of infectious diseases. But it is also worth noting the contributions of epidemiology in the investigation of non-infectious conditions.

From a methodological viewpoint, the three applied activities of epidemiology are, in nature, descriptive, analytical, and experimental. They may be used in a series of phases that include: a) the diagnosis; b) a description of the populations at risk and the temporal and spatial distribution of the disease. This may generate hypotheses; c) an investigative phase, which may involve a series of field studies designed to test these hypotheses; d) experiments performed under controlled conditions to test the hypotheses in more details; e) an analytical phase, in which the results produced by the above investigations are analysed. This may lead to modelling the epidemiology of the disease, which is useful to determine whether any vital information about the disease process has not been considered; f) possible interventions may also be investigated by measuring the impact of acting on specific disease determinants in small scale experimental or field studies; g) although presently rarely used in poultry, modelling can be a valuable tool for decision-making, allowing the exploration of various control measures; and, finally, h) a monitoring of the control measures to ensure that they are properly applied, and in order to assess their value.

Communicating Science-based Information to Non-scientists

Nathaniel L. Tablante

Virginia-Maryland College of Veterinary Medicine, University of Maryland College Park

Outbreaks of zoonotic diseases that have great economic and/or public health impact always get media and public attention. In a world dominated by social media and other electronic means of communication, disseminating objective science-based information to non-scientists such as the media, policymakers, and the public promptly and accurately is extremely important and helps ensure consumer trust and confidence, particularly in poultry and other food products derived from agriculture. For example, when outbreaks of catastrophic and economically devastating diseases such as avian influenza occur, our task as poultry veterinarians or poultry scientists is to communicate science-based information about the outbreak in language that is simple, clear, concise, and non-technical. The American Association for the Advancement of Science (AAAS) provides excellent tips on how scientists can communicate effectively with non-scientists. They suggest getting straight to the point, i.e. by explaining the "big picture" and why the audience should care, then getting into an appropriate level of detail to emphasize your points. It is important to think about your

audience and what they want to know rather than what you think they should know (they don't need a lecture on the molecular structure of avian influenza virus!). Always keep in mind that communication is a two-way process. Lastly, when communicating scientific information to non-scientists, two good acronyms to remember are KISS (Keep It Short and Simple) and BLUF (Bottom Line Up Front).

The One Health Movement: How Poultry Veterinarians Can be More Involved

Cheryl Stroud,

Executive Director, One Health Commission

Recently Avian influenza, AMR, Ebola, E coli, MERS, SARS, Salmonellosis, Zika etc. have emphasized the poignant need for a paradigm shift toward One Health thinking at all levels of academia, corporate, food production, lawmaking, public policy, and research systems. Additionally, the world must discover how to feed the projected 9 billion people postulated to populate its surface without causing further global destruction. Many One Health scientists and advocates believe that One Health is our 'Ray of Hope' for the future because they see our current ways of doing business in professional 'silos', with never a chance to interact directly to solve today's 'wicked' problems, as unsustainable. In addition to human and veterinary health specialists joining hands we need anthropologists, chemists, educators, engineers, private industry, social scientists, etc. to all move toward interactive One Health systems and thinking.

Many of us agree on these points. But what do we 'do' about it? This presentation will share a very brief historic context for global efforts to resurrect this very old concept and give an update on current happenings in the One Health 'movement'. Poultry veterinarians in all professional settings have a tremendous role to play in this movement, from being a first line of defense in recognizing and responding to emerging zoonotic diseases, to overseeing the safety of our food supply. We will discuss the critical need to connect the many passionate One Health stakeholders into conjoined efforts to address significant 'gaps' in our current systems and suggest One Health 'actions' that you, as poultry veterinarians might take to continue moving the needle forward for One Health.

Session A

Egg Production Drops in Brown Commercial Layers Investigated: Part 1

Kelli Jones[1], Eva Pendleton[2], George Boggan[1], Milos Markis[3], Jack Rosenberger[3]

*[1]Ceva; [2]Pennsylvania State University Animal Diagnostic Laboratory; [3]Aviserv*e

There have been recent reports of significant egg production drops in commercial brown layers in several areas of the United States. Initial observations suggest an early enteric component, as well. Production patterns will be shown related to this condition, along with discussions about early investigations into the potential causes of the egg production drops. Ultimately, higher than average reovirus titers, along with VN patterns similar to what is seen with the novel variant reoviruses sparked investigations into the potential role of reovirus in the egg production drops in brown flocks. Intervention strategies and prevention are discussed and the impact on egg production after these strategies were implemented are reviewed. This is one part of a multi part presentation.

Egg Production Drops in Brown Commercial Layers Investigated: Part 2

Eva Wallner-Pendleton[1], Kelli Jones[2], John Rosenberger[3], Milos Markis[3], Sherrill Davison[4], Donna Kelly[4], Patricia Dunn[1], H. Lu[1] and George Boggan[2]

[1]Pennsylvania State University, [2]Ceva, [3] AviServe, [4] University of Pennsylvania

Multiple cases involving commercial brown, young floor laying hens are described which were observed over a three-year period in Pennsylvania flocks. In general, most affected birds were either very slow to come into egg production or came into production and suffered a severe egg production drop. In many cases, the poor egg production persisted for several months and gradually came back up. Decreased feed consumption, failure to gain weight or weight loss as well as feed sorting and watery orange mucoid intestinal contents were seen. Gross and microscopic pathology, bacteriology, virology, and serology results are described.

Investigation of Egg Production Drops in Commercial Brown Layers: Part 3

Milos Markis[1], John K. Rosenberger[1], Sandra C. Rosenberger[1], Eva Wallner-Pendleton[2], Patricia Dunn[2], H. Lu[2], Sherrill Davison[3], Donna Kelly[3], Kelli Jones[4], and George Boggan[4]

[1] AviServe LLC
[2] Pennsylvania State University
[3] University of Pennsylvania, New Bolton Center
[4] Ceva Animal Health

Diminished egg production has been observed in multiple commercial brown layer flocks located in different geographical areas throughout the United States. Typically affected hens are floor-raised, cage-free and fed organic diets. Some flocks experience delayed onset of egg production whereas other flocks do not achieve peak production expectations. Enteritis and feed passage are frequently reported concurrent signs. Fibrosed digital flexor tendons have also been observed in some pullets and hens submitted for necropsy. Reovirus and adenovirus have been isolated from affected birds. Disease reproduction was attempted by inoculation of commercially sourced brown pullets/hens with a reovirus and adenovirus pool. Effect on egg production will be discussed.

A Unique Case of Ulcerative Dermatitis in a Flock of Cage Free, Commercial, Brown Layers

Geoffrey Lossie, Elizabeth Beilke, Eric Gingerich, Patricia Wakenell

Indiana Animal Disease Diagnostic Lab, Department of Comparative Pathobiology, Purdue University

A flock of cage free, Hy-Line brown layers was experiencing increased mortality. Ulcerative lesions above the tail head, with a concurrent increase in mortality, beginning at 25 weeks of age were the presenting clinical signs. A field visit was performed. At the time of the visit the total flock mortality was 12%. On site necropsy revealed numerous birds with ulcerative dermatitis and caseous peritonitis. Live birds were taken to the ADDL for necropsy. Gross necropsy revealed necrotizing dermocellulitis and myositis, fibrinous peritonitis, pericarditis, and splenomegaly. Histology revealed splenic and hepatic necrosis, serositis/airsacculitis, coccidiasis, and chornic/acute ulcerative, necrotizing dermocellulitis and myositis with feather follicle dysplasia. Bacterial culture of the skin revealed *Staphylococcus hyicus* and *Corynebacterium sp*. *E. coli* was cultured from

the liver/spleen. Moderate numbers of *Eimeria spp*. coccidia were noted on qualitative fecal flotation. The affected flock, and others belonging to the same company continued to see increased mortality with lesions identical to those described. Histopathology of the preening gland, and vitamin A levels were analyzed. Further diagnostic workup will include a small pilot study involving scarification of the skin and inoculation of the wound with the isolated *Staphylococcus hyicus*.

Bone deformities in Roller Pigeon squab due to improper diet.

Susan M Williams[1], Robert J Williams[2], and Robert M Gogal Jr[2].

[1]Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens
[2]Department of Veterinary Biosciences and Diagnostic Imaging, College of Veterinary Medicine, University of Georgia, Athens.

Several young squabs developed bone deformities during a breeding trial. In addition to bone deformities, chicks from the same breeding pair that hatched a couple of days apart, varied tremendously in weight gain, with the 2nd hatchling failing to thrive and dying within 9 days of hatching. Leg deformities were noticed at about 2 weeks-of-age. Birds were humanely euthanized and necropsied. Histopathology of the legs revealed nutritional deficiency (rickets). Analysis of the original commercial available diet, which had been successfully used in an adult study to simulate the natural diet, determined that there was 6% crude protein 4% crude fat, 6% crude fiber. The main ingredients are Milo, Cracked Corn, White Millet and Sun Flower Seeds. Initially, the treatment groups' squabs were affected. However, control birds also began to exhibit similar deformities. A new diet was implemented that used layer/breeder chicken feed, whole corn, feed wheat and chicken grit from commercial sources and mixed at the Poultry Diagnostic and Research Center. After the adult breeding pigeons were switched to a new higher protein diet, leg deformities and failure to thrive chicks no longer occurred.

Calcium tetany in flocks of commercial male turkeys?

H. L. Shivaprasad

CAHFS- Tulare Branch, University of California

Calcium tetany is a metabolic disease of various species of animals including birds due to hypocalcemia. This condition has not been reported in turkeys. Hypocalcemic tetany was diagnosed in two commercial male turkeys between the ages of 15 to 18 weeks based on low serum calcium levels. The turkeys were from a flock of 6000 and 8700 that experienced sudden onset of inability to walk, shaky legs, not eating and increased mortality ranging from 35 to 350 per day. Necropsy of 15 tom turkeys did not reveal any infectious diseases. Serum analysis from nine live turkeys reveled severe hypocalcemia. Supplementation of feed with oyster shells and vitamin D3 alleviated the situation.

Leucocytozoon sp. in a Flock of 4-week-old Ducklings

Richard M. Fulton, D.V.M., Ph.D.

Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University

A reportedly 7-week-old duckling, of undetermined type, was submitted dead for necropsy. The duckling, along with 6 other

flock mates, was purchased approximately three weeks previously. The week previous to submission 5 of the ducklings, like the one submitted, developed diarrhea and died within 24 hours of onset. Findings at necropsy were hepatomegaly (2x) and splenomegaly (15x). Microscopically, there were numerous megaloschizonts in the cerebrum. Small blood vessels of the brain and lung contained numerous leukocytes with sexual stages of a protozoal parasite. The spleen was expanded by large numbers of macrophages and mononuclear cells. Many splenic macrophages contained eosinophilic, granular, PAS-positive material within the cytoplasm and in occasional areas had hemosiderin. The liver had coagulative necrosis centered on central veins which was indicative of anemia. Based upon the morphology of the megaloschizonts, sexual stages within blood cells, and anemia related hepatic necrosis, a diagnosis of *Leucocytozoon* sp. infection was made.

Impact of Astrovirus Challenge on a Commercial Broiler Breeder Flock and Subsequent Progeny

David French[1], Phil Stayer[1], Erin Riley[1], Stanley Vanhooser[2], Pam Ferro[2]

[1]Sanderson Farms, Inc.
[2]TVMDL, Texas A & M University

A broiler breeder flock in Waco, Texas experienced a sudden drop in production at 29 weeks of age. The production drop ranged from 5 % to 20% by house. Onset was rapid, taking approximately 2 to 3 days and the drop in production lasted approximately 9 days before quickly returning to normal. Due to the rapid loss and regain of performance the incident was initially thought to be due to management issues and availability of water in the breeder houses. Mortality remained normal during the production drop and three were no apparent lesions in the breeder hens other than regression of ova. As the flocks were coming back into normal production mortality increased slightly. Birds were found to have large clutches of similar sized ova and the mortality pattern suggested the increase was due to calcium tetany and internal layers.

Three weeks after the production drop, hatchability dropped to 40% in this prime flock where fertility was previously determined to be 98% on these same eggs. Chicks that did hatch were small, obviously white in color rather than the typical yellow. Chicks that hatched had a brilliant green discoloration of the liver and in some cases had pale focal to multifocal plaques on the surface of the liver. Those that did not hatch were mostly dead embryos at 9 to 12 days of incubation. These dead embryos also had livers with the same obvious green discoloration. Tissues and serum were collected for diagnostics from the chicks that hatched as well as the chicks that did not hatch.

While virus isolation was initially unsuccessful, electron microscopy revealed what was identified by the lab as an Astrovirus. Histopathology and serology were also consistent with the diagnosis of an Astrovirus. Additional samples were collected at the hatchery, and sent to a different lab where the virus was finally isolated and introduced back into embryos in an effort to recreate the same lesions noted in our hatchery. The result was white chicks with the same liver lesions that were noted in the hatchery. Mortality at day 9 to 12 was not recreated as the embryos were older than that when the virus was inoculated into the eggs.

Incursion and Recursion of "White Chicks" in U.S. Commercial Broiler Production

Philip A. Stayer[1], Erin G. Riley[1], J. David French[1], P. Ferro[2], S. Vanhooser[2], A. Banda[3], B. Baughman[3]

[1]Sanderson Farms, Inc. [2]TVMDL [3]PRDL

Commercial broilers hatched as unusually "white chicks" have been reported from around the world, including the United States. One U.S. commercial broiler producer reported white chicks first in 2005 with recurring incidences up to the writing of this abstract. White chicks tend to be produced for 4 to 6 weeks, most often early in the egg production cycle, but not always limited to young hens. To date, elevated parent mortality has not been associated in hen flocks producing white downed progeny. Hen flocks producing white chicks may have no perceptible changes in production parameters up to severe drop in egg production. The onset of white chicks is typically associated with moderate to severe reduction in hatchability. Most of the lost embryos die during the second week of incubation with fibrotic livers. "Late dead" embryos, i.e., those lost in the last week of incubation, and day of age white chicks themselves tend to be smaller with larger unused yolk and green livers compared to unaffected hatch mates. This U.S. broiler producer utilized several diagnostic laboratories to characterize the histological lesions, virus identity and serology associated with "white chick disease". These laboratory reports as well as epidemiological links will be discussed.

Eimeria mivati: Field Clinical Case

Andres, Montoya, Steve Fitz-Coy, Doug Ward

Merck Animal Health, 2 Giralda Farms

Eimeria mivati, one of nine species of Eimeria known to cause coccidiosis in chickens, has been a source of controversy among poultry pathologists. Some believed it to be a distinct Eimeria species that posed a threat to broilers, but others have been doubtful and consider it either a variant of *E. acervulina* or a mixture of the *E. acervulina and E. mitis* species. It was first described in 1959 by Edgar and Siebold as a parasite of the upper small intestine. They noted that the parasite moved down the intestine as the infection progressed. With the shift from in-feed anticoccidials to vaccination of chickens for coccidiosis control, *E. mivati* has to be considered. It has been reported that *E. mivati* is pathogenic to chickens, resulting in impaired feed utilization, impaired growth and, sometimes, mortality depending on the level of challenge. A company in the Southeast of USA was experimenting loss in performance and some mortality. At necropsy gross lesions appear to be similar to *E. acervulina* but lesions extended to the midgut. Scraping for the midgut and observation under the microscope confirm the presence of *E. mivati*. In addition, tissue samples were collected for histopathology. In the past, we didn't have to deal with *E. mivati* before because anticoccidials were controlling it, but it's safe to assume that *E. mivati* resistance to anticoccidials may be developing just as it has for other Eimeria species.

Nasal Gland Chlamydiosis in Commercial Organic Turkeys in California: Association with Pigeons and *Chlamydia psittaci* Strain Characterization.

C. G. Sentíes-Cué[1], S. Carnaccini[1], M. Crispo[1], S. Stoute[1], H.L. Shivaprasad[2], R. Aaziz[3], K. Laroucau[3], and C. Corsiglia[4]

California Animal Health & Food Safety Laboratory System, [1]Turlock Branch, [2]Tulare Branch, School of Veterinary Medicine, University of California, Davis [3]Anses, Animal Health Laboratory, Bacterial Zoonoses Unit, Maisons-Alfort, France [4]Foster Farms, California

In October 2015, chlamydiosis causing nasal gland adenitis was diagnosed in a 13-week-old, commercial, free range organic turkey flock of 36,000 birds. This case was similar but unrelated to a case diagnosed in turkeys in the Central Valley of California in 2012. The only lesions involved were either unilateral or bilateral adenitis of the nasal glands and cellulitis in the upper periocular area. Chlamydia antigen was detected by both fluorescent antibody (FA) and immunohistochemistry in nasal glands, but not in the conjunctiva, spleen, and liver of the affected turkeys. Pigeons located at a feed mill located within a mile of the farm were positive for chlamydia by FA on conjunctival smears. Gross and microscopic pathology, FA, immunohistochemistry, PCR testing in the turkeys and pigeons affected, *Chlamydia psittaci* strain characterization, control measures, and discussion on probable factors involved with this outbreak will be presented.

Sep-Tox: Condemnation Without Representation

Armando Mirandé

Supervet, Inc.

Septicemia/Toxemia is one of ten classifications by USDA for whole bird carcass condemnation of broiler chickens, yet it comprises the majority, greater than half, of such condemnations at a processing plant (Agristats®).

Condemnations are mandated for those bird carcasses that do not meet the USDA's inspector criteria for wholesomeness and food safety, and/or are found to be adulterated. The official definition for adulteration would hardly justify any current broiler condemnation. Such definitions have not been updated despite extensive work done lately to incorporate new criteria and technologies to assess food safety.

Determining the causes in the field that lead to increased sep-tox condemnations is not always easy and, often, require more of a detective-like investigative approach and a complex understanding of the government's current political status towards consumer groups rather than a science/veterinary anamnesis of biological conditions leading to a septicemia-toxemia.

This case presents a sudden rise in sep-tox condemnations at a broiler integrator during a period of company's top performance parameters, including 7-day mortality, total cumulative mortality, average daily weight gains and feed conversion. Simply put the field situation and plant condemnations did not add up or made any sense. The presentation will focus in the systematic procedure a broiler production veterinarian can follow in order to correct, if at all possible, the biological events

leading to a sharp increase in sep-tox broiler condemnations while enjoying of an apparent healthy flock.

Ochroconosis and Rickets in Commercial Broiler Chicks

Natalie Armour[a], Brittany Baughman[a], Frank Austin[a], Danny Magee[a], Erin Riley[b], Phil Stayer[b] and David French[b]

[a]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University [b]Sanderson Farms, Inc.

Ochroconosis (Dactylariosis), caused by the dematiaceous fungus *Verruconis gallopava* (formerly *Ochroconis / Dactylaria gallopava*) is a sporadic fungal encephalitis of birds, with rare outbreaks reported in chickens. The present cases were diagnosed following reports of nervous signs in 14 and 16-day-old broiler chicks from two houses on a commercial six house farm. Mortality in the second week was 0.56%. Central nervous signs included ataxia, incoordination and tremors, with some chicks in lateral recumbency. Several chicks showed musculoskeletal signs, involving lameness with splayed legs. Cerebellar lesions including edema, congestion, hemorrhage and necrosis of varying severity were observed in 16 of the 22 chicks submitted for necropsy. Bone strength was reduced and the zone of hypertrophy of the tibiotarsal growth plate was widened in 5 chicks. Histologic examination confirmed granulomatous and necrotizing cerebellar encephalitis with GMS-positive intralesional fungal hyphae, while growth plate lesions were consistent with rickets caused by a phosphorus deficiency. *V. gallopava* was isolated from the brain tissue of chicks with cerebellar lesions, confirming Ochroconosis in these chicks. Additionally, *Escherichia coli* septicemias and *Eimeria maxima* and *E. acervulina* coccidiosis were diagnosed in several birds. Contaminated pine shavings are suspected to be the source of introduction of *V. gallopava* in this case.

Nutrition-Induced Respiratory Disease in Turkeys

Kabel M. Robbins

Butterball, LLC

This presentation will illustrate respiratory lesions and mortality associated with feeding turkeys a diet with elevated supplemental iodine. Mortality in flocks started at 12 days of age and continued for about 7 weeks. Affected turkeys developed lesions in the infraorbital sinus, nasolacrimal ducts, middle nasal chamber, trachea, and lungs. Mucus plugs located at the tracheal bifurcation were the identified cause of mortality. Airsacculitis was prevalent in flocks up to the time of processing. Epidemiologic investigation and extensive diagnostic sampling confirmed the absence of microbiologic causes. Lesions and clinical signs were reproduced in a feeding trial that compared 75 turkeys fed the test diet with 75 turkeys fed a standard diet. Chronic iodine toxicity is suspected as the cause of these lesions and mortality.

Histopathology of Nutrition-Induced Respiratory Lesions in Turkeys

Oscar J. Fletcher and Kabel M. Robbins

College of Veterinary Medicine, NC State University, Raleigh, NC 27607 and Butterball Corp.

This presentation will illustrate the histologic lesions associated with feeding turkeys a diet that was associated with increased mortality starting at 12 days of age and continuing for about 7 weeks. Affected turkeys developed

lesions in the infraorbital sinus, nasolacrimal ducts, middle nasal chamber, trachea, and lungs. Heterophilic, catarrhal, and lymphocytic tracheitis with squamous metaplasia were accompanied by plugs located at the tracheal bifurcation and consisting of mucus containing heterophils and bacteria. Lung lesions were catarrhal, heterophilic, and lymphocytic proliferative pneumonia expanding from secondary bronchi. Lesions were reproduced in a feeding trial that compared 75 turkeys fed the test diet with 75 turkeys fed a standard diet. Twenty turkeys from each group were examined by histopathology. Mucous plugs in nasolacrimal ducts and tracheal bifurcation were found only in turkeys fed the test diet. Lymphocytic and proliferative lung lesions were found in both groups. Chronic iodine toxicity is suspected as the cause of these lesions.

Variation in Gross and Histologic Lesions in Two Field Cases of Clade 2.3.4.4 H5 HPAI in British Columbia, Canada

Victoria Bowes

Animal Health Branch, BC Ministry of Agriculture, CANADA

History:

Case A: Flock of 6800 24-week-old broiler breeders with sudden onset of high mortality. Farm location is within 3 km of an active outbreak of H5N2 HPAI.

Case B: Flock of 60 free-range 1.5yr Isa Brown laying hens with high mortality. Farm location is 20km from a 6-week previous H5N2 HPAI outbreak premises.

Necropsy:

Case A: Birds are in good general body condition and are actively in lay. Gross lesions include severe facial edema, intense conjunctival hyperemia, severe bilateral pulmonary congestion & edema (lungs sank in formalin), petechial hemorrhages in the epicardium & parietal pleura, a friable, congested liver & spleen and superficial cecal tonsil hemorrhage.

Case B: Birds are in good general body condition. Gross lesions include mild cyanosis, hemorrhagic shanks, necrotizing conjunctivitis, diphtheritic pharyngitis/proximal esophagitis, multifocal miliary splenic necrosis and bilateral pulmonary hemorrhage.

Diagnostics: Both cases were positive for AI Matrix & H5 rRT-PCR (multiple tissues), HA sequencing performed at NCFAD conformed with the OIE definition of HPAI and genetic sequencing determined Case A to be H5N2 clade 2.3.4.4 and Case B to be H5N1 clade 2.3.4.4..

Histopathology:

Case A: Eyelid: There is marked generalized conjunctival congestion with mild generalized infiltration of the peripheral epithelium with small aggregates of heterophils. There is generalized random multifocal subepithelial acute perivascular necrosis with extravasation of acute inflammatory cells. There are occasional random foci of lymphocytic aggregates. Trachea: There is moderate generalized submucosal congestion with occasional random foci of submucosal perivascular necrosis with infiltration by heterophils and lymphocytes. The ciliated epithelium is intact.

Case B: Eyelid: There is marked generalized conjunctival congestion and edema. There is severe generalized acute multifocal epithelial and subepithelial necrosis with infiltration by heterophils and lymphocytes. Trachea: There is marked generalized submucosal congestion with acute multifocal mucosal necrosis and infiltration with mixed inflammatory cells. There is significant epithelial attenuation and loss. There are occasional dense submucosal perivascular lymphocytic aggregates

(vasculitis). Adjacent esophagus displays multifocal acute epithelial necrosis with infiltration by heterophils.

This case illustrates the subtle differences in tissue tropism and the nature of the gross & histologic lesions between HPAI viruses with minor genetic variation. The diagnosis of HPAI in poultry is primarily based on a history of catastrophic mortality in susceptible birds, compatible gross lesions and molecular diagnostics. Histopathology is not specific although often spectacular.

*presented as a case report at the Western Conference of Veterinary Diagnostic Pathologists, Sept 25-26, 2015, Abbotsford, BC

Pathology Associated with Highly Pathogenic H5N8 Avian Influenza in Commercial Chickens and Turkeys in California

Simone Stoute[A], Richard Chin[B], Beate Crossley[C], C. Gabriel Sentíes-Cué[A], Arthur Bickford[A], Mary Pantin-Jackwood[D], Richard Breitmeyer[C], Annette Jones[E], Silvia Carnaccini[A], and H. L. Shivaprasad[B]

California Animal Health & Food Safety Laboratory System, UC Davis, [A]Turlock Branch, [B]Tulare Branch [C]Davis Branch, [D]Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, U.S. Dept. of Agriculture, Agricultural Research Service, [E]California Department of Food and Agriculture, Animal Health and Food Safety Services

In January 2015, highly pathogenic Eurasian lineage H5N8 avian influenza (AI) virus was detected in commercial meat turkeys in Stanislaus County, California. Approximately 3 weeks later, a similar case was diagnosed in commercial chickens from a different company located in Kings County, CA. Five, 14-wk-old turkey hens were submitted to the California Animal Health and Food Safety Laboratory System (CAHFS), Turlock and eleven, 12-wk-old chickens were submitted to CAHFS, Tulare laboratory due to an acute increase in flock mortality. Gross lesions included enlarged and mottled pale spleens and pancreas in turkeys and chickens. Histologically, the major lesions observed in turkeys and chickens were splenitis, pancreatitis, encephalitis and pneumonia. Immunohistochemistry (IHC) performed on various tissues from both cases indicated a widespread AI virus tissue distribution. Except for minor variations, the tissue distribution of the AI antigen was similar in the chickens and turkeys. There was positive IHC staining in brain, spleen, pancreas, larynx, trachea and lungs in both chickens and turkeys. Hearts, ovaries and air sacs from the turkeys were also positive for AI antigen. The liver sections from the chickens had occasional AI positive staining in mononuclear cells but the IHC on liver sections from the turkeys were negative. The bursa of Fabricius, small intestine, kidney and skeletal muscle sections were negative for AI antigen in both chickens and turkeys. The AI virus from both cases was 99% identical to an H5N8 AI virus (A/gyrfalcon/Washington/41088-6/2014) isolated from a captive gyrfalcon from Washington State in December 2014.

Predation of a Flock of Mallard Cross Ducklings

Richard M. Fulton, D.V.M., Ph.D.

Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University

Mallard cross ducklings were purchased for an 86-year-old woman to enjoy since she had recently lost her husband. According to a

family friend, the daughter had threatened to kill the ducks among other things and a restraining order against the daughter was obtained. The ducks were allowed in a fenced yard during the daytime and kept in a locked building at night. One morning, the family friend found the ducks piled upon one another. It appeared as though someone had killed them, piled them up, and had tried to cut off the head of two of the ducks. All five ducklings were submitted for necropsy. One carcass was missing a head and another disjointed head was found in the box of ducks. The remainder of the ducks had various amounts of skin and feathers removed from the dorsum of the head and proximal neck. There was dried blood on the skin and feathers as well as underneath the skin. Some of the neck muscle was tattered and missing. One duck had a transected trachea and another had a fractured mandible. Close examination of the skulls of the ducks revealed the presence of two puncture wounds approximately 5 mm apart. Based upon gross lesions and pattern of lesions, a diagnosis of predation was made. The most likely predator was a weasel, marten or mink. Methods of kill and physical evidence of predation by different predators will be reviewed.

Investigation on the use of a serological DIVA monitoring strategy when a rHVT-F vaccine is used to control Newcastle disease.

Yannick Gardin[1], Vilmos Palya[2], Timea Tatar Kiss[2], Stephanie Lesceu[3], Marcelo Paniago[1], Pascal Paulet[1], John Elattrache[4], Sjaak De Wit[5]

[1]Ceva Santé Animale, France, [2]Ceva Animal Health, Hungary, [4]IDvet, France, [4]Ceva Animal Health, United States, [5]GD Deventer, The Netherlands.

Vaccination against Newcastle disease (ND) is routinely applied in many countries, either because the disease is strongly present or because the risk of being infected is considered as too high and / or the consequences too costly.

Controlled experiments as well as field experience have demonstrated the capacity of an rHVT-F ND vaccine to induce solid protection, so that this rather new category of vaccine has gained strong and world wide acceptance. It is now widely used, either as a standalone vaccine solution or in combination with other ND vaccines including live, and sometimes also killed, vaccines.

In all countries where ND vaccination is used, there is interest in assessing the actual take of the vaccines and eventually monitoring the infection, if present, using serology. This is particularly true in countries where ND is not (or not strongly) present and where the rHVT-F vaccine is used exclusively. Because it contains no live or killed ND virus, corresponding humoral immune response allows for a DIVA monitoring strategy.

Several studies including challenge studies have been conducted with various serological assays and molecular tools to assess the

"take" of the vaccine and detect the field infection.

These results will be presented and discussed.

A new enteric disease in Minnesota turkeys caused by picorna- and picobirnaviruses?

Sunil K. Mor,[1] Rosemary A. Marusak,[2] Robert E. Porter,[1] and Sagar M. Goyal[1]

[1]Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine
College of Veterinary Medicine, University of Minnesota
[2]Sanofi Pasteur- Viral technology

Since August 2014 the University of Minnesota-Veterinary Diagnostic Laboratory has received cases related to a new enteric disease problem in Minnesota turkeys. The disease in turkey flocks is characterized by poor performance, poor uniformity, reduced feed conversion and reduced market weights. These flocks develop a dark, foul-smelling diarrhea starting at 8-10 weeks of age, which continues through age 15-16 weeks. In later stages of infection, the turkeys pass undigested feed in relatively well-formed feces. The weight of infected birds is reduced by ~2-3 lbs as compared to their standard breed character. Morbidity varies from flock to flock and in some cases reaches 100%. At necropsy, gross lesions are seen only in the intestines. Undigested feed with increased mucus was observed in intestines along with prominent mucosal congestion/hemorrhage. Lymphocytic infiltrates expand the villi to form lymphoid follicles, which are sometimes accompanied by heterophils in duodenum and jejunum. In a preliminary molecular study, we tested pools of fecal samples from apparently healthy (16 pools) and affected birds (30 pools) and found a high viral load in the feces of affected birds as compared to apparently healthy birds from the same flock. Interestingly, picobirnavirus was detected only in affected birds (66.7%; 20/30 fecal pools). Analysis of a pool of feces from affected birds by next generation sequencing (Illumina Miseq) identified genetic sequences corresponding to picobirnavirus, a novel picornavirus, a recombinant of turkey and chicken picornaviruses, and commonly known turkey picornaviruses of genra *Megrivirus* and *Gallivirus*. We believe that turkey picobirnavirus, alone or with these novel picornaviruses, may be a cause of this new type of turkey enteritis.

Phylogenetic analyses of diverse, novel enteric picornaviruses detected in turkeys and chickens in the United States.

J. Michael Day

Southeast Poultry Research Laboratory

Investigations in our laboratory utilizing the next-generation of high-throughput nucleic acid sequencing technology and subsequent viral community analysis—an approach called viral metagenomics—have revealed several novel intestinal ribonucleic acid (RNA) viruses in poultry. Of particular interest, our comparative metagenomic analyses of the viruses present in the intestines of healthy versus birds suffering from enteric disease have identified novel picornaviruses—small viruses with RNA genomes—that appear to be associated with the appearance of enteric disease in the field as well as under controlled experimental conditions. The picornaviruses are members of the Family *Picornaviridae*, which is part of the larger *Picornavirales* Order, and includes viruses that infect and cause disease in many different groups of animals. In fact, several community-based investigations from around the world have

indicated that the picornaviruses are common constituents of the avian gut. In order to gain a better understanding of the prevalence and molecular phylogenetics of the poultry enteric picornaviruses, we designed molecular assays targeting the enteric picornavirus *3D^{pol}* (RNA-dependent RNA-polymerase) and the *VP3* capsid genes. Our sequence analyses have indicated that the enteric picornavirus generally cluster based upon species and geographical origin, although some may have crossed the species barrier. A discussion detailing the efforts to place these novel poultry enteric picornaviruses within the rapidly expanding taxonomy of the *Picornaviridae* will be presented.

Prevalence of parvovirus in Minnesota turkeys

Tamer A. Sharafeldin, Sunil K. Mor, Mostafa Y. Abdel-Glil, Azad Singh, Sagar M. Goyal

Department of Veterinary Population Medicine, Minnesota Veterinary Diagnostic Laboratory University of Minnesota

Poult enteritis Syndrome (PES) is characterized by enteritis and decreased body weight gain in growing turkey poults between 1 day and 7 weeks of age. Another syndrome called the light turkey syndrome (LTS) causes a decrease in body weight of adult tom turkeys in Minnesota leading to huge economic losses. Reovirus, rotavirus, and astrovirus are considered to be involved in LTS flocks in Minnesota. In order to determine if a DNA virus might be involved in these cases, we tested 115 fecal sample pools collected from four LTS flocks and two non LTS flocks in Minnesota for the presence of parvovirus. In addition, 116 turkey fecal and meconium samples submitted to Minnesota Veterinary Diagnostic Laboratory were tested for the presence of parvovirus. The samples were tested by PCR using primers for the non-structural 1 (NS1) gene. From 80 samples of LTS flocks, 41 were positive for parvovirus and 20 samples from the 35 non LTS flocks samples were positive. The prevalence of parvovirus in submitted fecal samples was relatively low; only five of 116 PES pools were positive. Partial gene sequence of NS1 gene suggested that the virus detected in our study was closely related to the previously described parvoviruses from turkeys. This study reports the prevalence parvovirus in LTS and Non-LTS turkey flocks in Minnesota.

Does Early Exposure to *Clostridium perfringens* Provide Some Immunity to a Severe *Clostridium perfringens* Oral Challenge at 17 Days in a Necrotic Enteritis Challenge Model?

Stephen Davis, S. Hendrix, D. Moore

Colorado Quality Research, Inc.

It has been reported from commercial broiler field cases of necrotic enteritis, that cases occurring in new farms or recently cleaned out farms with new litter, experience more severe cases of the disease compared to broiler flocks that are placed on the same farms or other farms with used litter in the brood area.

Floor pen studies were conducted to compare the severity of necrotic enteritis (NE) lesion scores and mortality when day old broiler chicks were placed on used litter from a prior Clostridium perfringens challenge study versus day old broiler chicks placed on new clean pine shavings and both groups received the same controlled severe oral challenge at 17 days.

Results, discussion and conclusions from these studies will be presented with consideration with and without day old coccidiosis vaccination and/or coccidiosis contamination.

Necrotic Enteritis in turkeys in California and characterization of *C. perfringens* isolates for toxins

H. L. Shivaprasad, G. Senties Cue, S. Stoute, S. Carnaccini, N. Mishra and J. Smyth.

CAHFS- Tulare and Turlock Branches, University of California, Davis, CA and University of Connecticut

Necrotic enteritis (NE) is an acute bacterial infection primarily of the intestine and occasionally of the liver in chickens and turkeys caused by *C. perfringens.* There has been an increased incidence of NE in turkey flocks in California ever since the flocks have been raised free of antibiotics (ABF) including ionophores. Approximately 60 isolates of *C. perfringens* isolated from the intestine of turkeys are being tested for various toxins including alpha, netB, TPE L, beta 2 and other enterotoxins. Data on the incidence of NE in turkeys in the pre and post ABF years as well as the toxin types detected in the *C. perfringens* will be presented and discussed.

Necrotic enteritis: The Struggles Understanding This Syndrome

Steve Fitz-Coy

Merck Animal Health

Necrotic enteritis is an acute enterotoxemic condition of young chickens and turkeys, often associated with high sudden mortality for a short duration. This syndrome was first reported in 1961 from a flock of cockerels in England. Since then, this disease has become relatively prevalent in global commercial poultry production. With increasing pressures from customers and escalating cost of production, companies have made modifications in commercial poultry management practices. Accompanying some of these changes are enteric challenges in which necrotic enteritis is relatively common. Necrotic enteritis was seen in chickens as young as six day of age and without any coccidia association. The necrotic lesions were seen in the lower small intestines and sometimes include the large intestine and ceca. Sometimes, the lesions seen were not consistent with the *"Turkish Towel Syndrome"* - the tissue and content appeared to be in a liquefied state referred to as "liquefactive necrosis". The lesions were very seldom at or above the yolk stalk. These lesions were seen in samples from across several companies and states across the USA.

Future of Blackhead Disease in Poultry

Steven Clark, DVM

Devenish Nutrition

A turkey health survey[1] of US veterinarians in turkey production ranked blackhead position #13 (#11 prior year) compared to #22 in 2006; the survey ranked and scored 36 current disease issues (1= no issue to 5 = severe problem) and had a survey response (reply) of 100% (n=25). Blackhead, also known as Histomoniasis, is one disease with no efficacious drug approved for use in turkeys. There were 55 reported cases of blackhead (2015) an increase from 61 the prior year, and a record 108 in 2010. Histomoniasis occurs regionally and seasonally in turkeys, and can result in significant mortality. Dimetridazole was extremely efficacious and previously approved for use in turkeys for the prevention and treatment of blackhead; it was banned in 1987. The lack of any legal treatment for histomoniasis is of concern, especially in the case of valuable turkey breeder candidate flocks. Losses to blackhead have been severe in several areas of Europe, and sporadic

cases are occurring in North America. On April 1, 2015, the sponsor announced that it would discontinue marketing nitarsone, by fall 2015, and would request withdrawal of the approval for the drug by the end of 2015. The approval of all applications for use of nitarsone in animal feed were withdrawn as of December 31, 2015; following this action, there are no FDA-approved, arsenic-based drugs for use in food producing animals.[2] Nitarsone was FDA approved for the prevention of histomoniasis (blackhead disease) in turkeys and chickens, and was the only approved animal drug for this indication. Nitarsone will cease to be available in the 2016 growing season. There are neither effective nor approved products for treatment of blackhead outbreaks. Under the announced FDA phase-out plan, the strategy allowed affected producers the opportunity to consider alternatives for managing this disease in the future.

Blackhead Disease, also known as Histomoniasis, is caused by *Histomonas meleagridis*, a flagellated protozoan. Classically indirect transmission is accepted, whereas the bird ingests embryonated ova of cecal worms (*Heterakis* gallinarum) that contain the *Histomonas* organism. *Histomonas meleagridis* is pleomorphic; in the cecum *Histomonas* is flagellated whereas in tissues (cecal wall, liver) it is a strictly amoeboid organism. *Heterakis gallinarum*, also known as cecal worm of poultry, acts as an intermediate host. A new parasitic cycle starts with the intake of ceca-dwelling forms of the protozoa *Histomonas meleagridis* by adult cecal worms in the ceca of the turkey. New research and field observations support the opinion that direct transmission is possible as well where the *Histomonas* are able to infect turkeys for a short period after being excreted. Since oral transmission seems impossible, it has been demonstrated that the mechanism for this is “cloacal drinking”[3]. Dr. McDougald suggests that the *Histomonas* protozoa in the feces, directly infects new turkeys via the cloacal route, and can spread rapidly through the flock.

Histomoniasis in turkey flocks before and after the ban of nitarsone

Rüdiger Hauck[1], H. L. Shivaprasad[2]

[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis [2]California Animal Health and Food Safety Laboratory System, Tulare Branch, University of California Davis

Information about the cases of histomoniasis diagnosed at the California Animal Health and Food Safety Laboratory System and other laboratories in USA before and after the ban of nitarsone will be presented.

These results will be compared with the experience in Germany after 2003, when the ban of nifursol by the European Union took effect. Nifursol had been the only antihistomonal compound left after the ban of nitroimidazoles several years before. Like nitarsone, it was to be used prophylactically as feed additive. While in the USA in spite of the availability of nitarsone cases of histomoniasis have been reported occasionally in recent years, histomoniasis was extremely rare in Germany before 2003. Since then, the disease has been diagnosed frequently in commercial turkey flocks. While in some cases, the disease could be contained by separation of the houses into compartments and a quick change of litter or transferring the birds to other houses, often whole flocks were euthanized. In spite of anecdotic reports of the usefulness of plant compounds to prevent histomoniasis, their use often did not prevent outbreaks of the disease.

A Recurrence of Blackhead in Young Turkey Breeder Toms in a House that had Experienced Blackhead Many Years Previously

Eric Gonder, Becky Tilley

Butterball LLC

A flock of 16-week-old turkey breeder toms experienced over 80% mortality within a month due to blackhead, despite administration of anthelmintics and arsenicals. The house involved had experienced an outbreak many years before following replacement of the clay floor with new clay, probably contaminated with heterakis eggs. Many flocks had been raised in this house between the outbreaks with no clinical signs of blackhead.
We speculate that the clay floor had deteriorated enough to again expose viable histomonad-contaminated heterakis eggs, precipitating the current outbreak.

Experiences Treating Histomoniasis in Turkeys

Brian Wooming VMD

Cargill Turkey & cooked Meats

Histomoniasis has been an infrequent problem in our turkey complexes, a fact that has been attributed to the use of Histostat in feed. 2015 was a particularly difficult year, both in terms of the frequency of the problem as well as the severity of some of the cases. This report will describe four cases to provide some insight into the geographical and clinical variation at presentation. The cases will be selected to depict the different interventions tried as well as the observed response.

Field Experiences Controlling Histomoniasis Blackhead Disease in Turkeys at a Large Multi-Age Commercial Growout Farm

Arun K. Bahl

Bahl Farms Inc./Consulting

Most of the significant research on blackhead disease in poultry was conducted in the early 1970's when highly effective chemotherapeutic agents were available. The last available and effective drug, Histostat (Nitarsone, 4-Nitro, 4-Nitrophenylarsonic Acid), currently is the only approved product for preventative use, but effective end of year 2015 is being taken off the US market. There is no effective approved therapeutic available to control the disease. Histomoniasis appears to be a re-emerging issue and can infect many poultry species. Turkeys, in particular are very susceptible to the disease. A novel approach was taken in an effort to slow down or eliminate the grave losses experienced repeatedly at a large multiage tom turkey growout facility. High morbidity and continued mortality rates of eighty percent were experienced in the affected barns. Isolation of Histomonas meleagridis, at University of Georgia, and subsequent experimental reproduction confirmed the strain to be highly virulent. Complex epidemiology of Histomonas was considered and evaluated with an approach to contain or eliminate the disease from reoccurring. Suggested and implemented changes included litter management, treating the built-up litter base, the outside perimeter of the barn for nematode control, limited use of Histostat in the brooder barn diets, essential oils (oil of oregano) inclusion in all the feeds, use of fecal and litter moisture control natural products and overall sanitation and management. Blackhead has not been an issue for the past almost three years. Histostat will not be used in the brooder

barn diets effective January 1, 2016. Information presented will include total farm performance.

Effects of Feed Additives on The Progression of Blackhead Disease in Turkeys

Robert B. Beckstead and Miguel A. Barrios

Poultry Science Department, The University of Georgia
Jefo Nutrition, Inc., Canada

The removal of Histostat from the market has resulted in the need for alternative preventatives and/or treatments for Blackhead disease. Numerous feed additives have been suggested as having antihistomonal properties by inhibiting *Histomonas meleagridis* growth, enhancing gut health or improving innate immunity. The objective was to test different commercially available feed additives in an *in vitro H. meleagridis* cell screen, as well as, a direct and lateral Blackhead disease model. A cell screen was developed using cell culture flasks and Dwyer's media. All flasks contained 100,000 cells and after 24 hours of growth at 42^{o}C, cells were treated with 6 different concentrations of the compounds. Cells were counted after 8 and 40 hours of incubation using a Neubauer haemocytometer. Treatments included an essential oil extract, a phytogenic feed additive, an organic acid, essential oil blend, and a yeast derivative. For the second part of the study, 10 days of age poults will be divided into treatments including a positive and negative control with and without challenge. There will be 30 birds per treatment and 3 replicates per treatment for a total of 540 poults. Treatment diets will be fed and poults will be challenged with a field strain of *H. meleagridis*. After 10 days, poults will be scored (0-4) for Blackhead lesions. The results of this study will be useful in determining if these feed additives are viable treatment strategies for Blackhead disease.

Geospatial Mapping of Histomoniasis Outbreaks in Commercial Poultry in the Southeast United States

Rebecca E. Jones[1], David V. Rives[2]

1. Poultry Health Management Program, Department of Population Health and Pathobiology, North Carolina State University, 2. Zoetis, Inc

We evaluate a commercial turkey integrator in the Southeast US with an increased farm incidence of histomoniasis of approximately 30% as compared to 6-7 years prior. This increased incidence has so far proven inconsistent with the spread of *Heterakis gallinarum* via lapses in biosecurity of personnel, yet geographically overlapping turkey integrators are not reporting a similarly increased incidence of histomoniasis. Flock global positioning system (GPS) and mortality data associated with histomoniasis outbreaks over the past decade are currently under analysis for the affected integrator, while collection of similar data from surrounding broiler breeder operations is underway. Our goal is to overlap this data using geographical information systems to determine the pathogenesis of increased incidence of histomoniasis within the affected integrator.

The Clinical and Economical Impacts of Blackhead in Broiler Breeder Pullets

Deirdre Johnson, Don Ritter

Mountaire Farms; Breeder Complex

Mountaire Farms experienced increased clinical Histomoniasis in multiple pullet rearing houses in the spring and fall of 2015. The breaks occurred between 2-5 weeks. They

presented with increased mortality and the classic typhlitis and liver lesions. The presentation will include a brief explanation of the life cycle of Histomoniasis followed by a more in depth discussion of responses to multiple treatments.

The second part of the presentation explores the economic impacts of Histomomiasis, which extend well beyond rearing mortality. Because of the pathology that occurs in the ceca and liver, pullet development is impaired. Increased uniformity and optimal body weight maturedness are critical to a pullet flock developing into a productive breeder laying flock. This presentation includes analyses of coefficients of variation, body weights, mortality, and egg production of the affected flocks versus unaffected flocks.

Investigating the prevalence of *Histomonas meleagridis* shedding by captive raised Ring-necked pheasants (*Phasianus colchicus*) in Pennsylvania

Richard Gerhold[1], Kate Purple[1], Mabre Brand[1], Justin Brown[2], and Robert Boyd[2]

[1]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, The University of Tennessee [2]Pennsylvania Game Commission

Ring-necked pheasants (*Phasianus colchicus*) were introduced into the United States including multiple regions in Pennsylvania, producing substantial hunting opportunities. Pheasants can harbor multiple pathogens in the absence of overt disease including *Histomonas meleagridis*, which is considered to be one of the most important pathogens for native game birds including Wild turkey (*Meleagris gallopavo*), Ruffed grouse (*Bonasa umbellus*), and Northern bobwhite (*Colinus virginianus*). In addition, *H. meleagridis* has caused significant impacts in the commercial turkey industry. The Pennsylvania Game Commission annually raises and releases about 200,000 pheasants from 4 game farms, and as a responsible wildlife management agency, was interested the infection status of *H. meleagridis* in pheasants. Fifty-one pheasants from a single game farm were examined for *H. meleagridis* by inoculating cloacal swabs into flasks containing Dwyer's media. Flasks were shipped to the University of Tennessee using a published protocol for survival of *H. meleagridis* in transit. Flasks were examined daily for seven days for histomonad growth. In addition, an aliquot from twenty randomly chosen flasks was used for DNA extraction and PCR targeting the internal transcribed spacer (ITS) regions of the ribosomal RNA. All flasks were culture-negative via light microscopy and DNA extract from the twenty samples was PCR-negative. These data suggest that Ring-necked pheasant propagation facilities may not be as important in the ecology of *H. meleagridis* as previously suspected. More research is needed to examine the frequency of *H. meleagridis* shedding in pheasants and other species to further understand the eco-epidemiology of blackhead.

Overview of field and experimental experiences with paromomycin as treatment for histomonosis in turkeys

Koen De Gussem, Wouter Depondt, Vereecken Monita

Huvepharma NV, Belgium

Turkeys are by far the most susceptible species to histomonosis. Due to increasing regulatory restrictions no prophylactic or therapeutic drugs against histomonosis were available for food producing animals on the European market during the last decade. This has recently changed with the registration of

Paramomycin 200 g/1000 g in Italy, a product containing the active paromomycin, registered for the treatment of histomonosis. Paromomycin is an aminoglycoside and is widely used and authorized to treat some protozoal infections. In *Histomonas meleagridis*, the Mode of Action for paromomycin has been unraveled (1). In challenge studies, performed with different *Histomonas meleagridis* strains, supplementation of paromomycin resulted in a significant decreased mortality and turkeys in treated groups had significant lower scores for liver and caecal lesions compared to untreated challenged control birds. Also field studies and practical experiences, using the product when outbreaks of Histomonas occur, demonstrated the efficacy of the product. Application in an early stage of infection is necessary, since paromomycin is poorly absorbed from the gastro- intestinal tract. This is why early diagnosis of histomonosis, by means of monitoring of mortality, necropsy, histology and PCR, is crucial. Besides supplementation of paromomycin, attention must be paid to management of the affected farm. A good hygiene and litter management will contribute to decrease the spreading of the parasite in the flock.

Bleyen et al. Veterinary Parasitology 165 (2009). p248-255. Non-curative, but prophylactic effects of paromomycin in Histomonas meleagridis-infected turkeys and its effect on performance in non-infected turkeys

A clonal culture as Key tool to develop new diagnostics and a vaccine against Histomonosis

M. Hess

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Histomonas meleagridis is a flagellated protozoan parasite inducing histomonosis in poultry. Turkeys are most severely affected and mortality can reach up to 100%. At certain stage depopulation of the whole flock has to be performed to reduce suffering of animals, underlining the impact of the disease on animal welfare. In chickens the infection is less severe, but outbreaks with increasing mortality and production losses are also reported. Specific prevention or therapy is no longer possible within the EU and the USA due to the ban of all effective chemotherapeutics. As a consequence, reports about clinical outbreaks increased in recent years.

Isolation and cultivation of the parasite is far from being a routine procedure due to the low tenacity of the parasite and the requirements for the substrate, e.g. the presence of bacteria in the culture medium. Furthermore, other protozoa present in the gut can complicate the isolation. The establishment of clonal cultures offered numerous advantages to study the biology of *H. meleagridis* in greater detail, applying a well-defined setting. Furthermore, new diagnostics, like PCR, ELISA, in situ hybridization and immunohistochemistry, were developed. Long term *in vitro* cultivation resulted in attenuation of the parasite for turkeys and chickens. Furthermore, birds infected with these parasites were protected against homologous and heterologous challenge. Backpassages, in turkeys and chickens, demonstrated robust attenuation with the absence of clinical signs and lesions mainly confined to the caeca of turkeys.

Altogether such data emphasize the value of clonal cultures for a vaccination strategy against histomonosis.

The History of the First Generation Marek's Disease Vaccines: The Science and Little Known Facts.

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Shortly after the isolation of Marek's disease (MD) herpesvirus (MDV) in the late 1960's vaccines were developed in England, USA and The Netherlands. Biggs and associates at the Houghton Poultry Research Station (HPRS) in England attenuated HPRS-16, the first cell culture isolated MDV strain, by passaging HPRS-16 in chick kidney cells. Although HPRS-16/Att was the first commercial available vaccine, it never became widely used and was soon replaced by the FC126 strain of herpesvirus of turkeys (HVT) vaccine developed by Witter and associates at the Regional Poultry Research Laboratory [now Avian Disease and Oncology Laboratory (ADOL)] in East-Lansing, Michigan. Ironically, Kawamura et al) isolated a herpesvirus from kidney cell cultures from turkeys in 1969 but never realized its potential as a vaccine against MD. Rispens of the Central Veterinary Institute (CVI) developed the third vaccine. His associate, Maas, had found commercial flocks of chickens with MDV antibodies but without MD. Subsequently, Rispens isolated a very low pathogenic strain from hen number 988 from his MD antibody-positive line 7 flock. This isolate became the CVI-988 vaccine used mostly in The Netherlands. During the late 1970's HVT did not longer fully protect. The addition of SB-1, isolated by Schat and Calnek, to HVT improved protection against the emerging very virulent strains. In the 1990's CVI-988 became the world-wide vaccine gold standard. This review will present data from published papers and personal communications providing additional information about the exiting 15-year period after the isolation of MDV to the development of the different vaccines.

Coccidiosis Vaccination Revealed: A Peek Behind the Curtain

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Coccidial vaccination is one of the choices available to control coccidiosis in commercial broilers. These live oocyst products are often used as part of a rotational program schedule with anticoccidial drugs and chemicals. Use of coccidial vaccines for a few broiler growing cycles annually may prolong the efficacy of drugs and chemicals and provide a more sustainable long term strategy for optimal control of coccidiosis. One large drawback to achieving consistently successful results with coccidial vaccines in broilers is how to best determine if the product being used contains sufficient numbers of LIVE oocysts to immunize the birds. Surprisingly, this topic is rarely discussed in a public setting. Another significant hurdle to successful use of coccidial vaccines is knowing how to best administer the vaccine. To answer these questions coccidosis vaccines from major suppliers were evaluated and compared for viability and consistency. Different vaccine application methods using coccidial vaccines from major suppliers were similarly compared. Detailed methods and comparative results of these evaluations will be shared.

Comparison of Breeder/Layer Coccidiosis Vaccines

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In recent years, there have been several coccidiosis vaccines introduced to the broiler market by multiple manufacturers. The international vaccine options for breeders and layers, namely, those that include *E. brunetti* and *E. necatrix*, have been limited to only one or two vaccines in a given market. Recent vaccine introductions in Europe have expanded the number of internationally available breeder and layer vaccines. Five breeder and layer vaccines were compared for their potential pathogenicity at extra-label high dose in pen studies. The high dose scenario is created under field conditions when application errors result in naïve birds mixed with properly vaccinated birds. As properly vaccinated birds begin to shed oocysts, the naïve birds will be exposed to a high dose of vaccine-type oocysts shed from the vaccinates. The vaccines were also evaluated for their relative precocity (reduced prepatent period). Precocious strains lose one or more multiplicative asexual stages in the Eimeria life cycle, resulting in both a reduction of prepatent period and a reduction in pathogenicity. Greater precocity is equated to a reduction in tissue damage and oocyst shedding (attenuation). The attenuated vaccines from Europe were further tested to determine the onset of immunity based upon challenge at weekly intervals with mixed *Eimeria spp.,* including *E. necatrix* and *E. brunetti*.

Evaluation of Gel Application of a Coccidia Vaccine

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Coccidiosis is an economically significant enteric disease of chickens that is caused by coccidia, a parasitic protozoan of the *Eimeria* species. Vaccination against coccidia has become more prominent of late because multiple external factors have affected other control methods that prevent disease. Traditionally, vaccines for coccidiosis are administered in the hatchery at one day of age via spray cabinet to produce liquid droplets for ingestion of oocysts. However, a new gel delivery system is being used with increasing frequency where a straight application bar streams coccidia vaccine mixed with a highly viscous gel onto the chicks. The idea is that the gel beads will remain intact on the chicks longer than a traditional liquid spray bead, increasing time for preening and ingestion of oocysts in the vaccine. To date, no formal research has been done to evaluate the efficacy of this new gel delivery system, or to compare this new method with spray application. The aims of this study were to evaluate the effectiveness of both spray and gel application methods for coccidia vaccination, as well as compare both methods to oral gavage. For each vaccinated group oocyst shedding was recorded and birds were challenged with *Eimeria maxima* oocysts. Oocyst shedding was monitored for 7 days post-challenge until necropsy, and body weight gains, gross and microscopic lesions, and protection levels from challenge were

evaluated. The results from this study will provide valuable insight for the poultry industry, as currently there is no formal research on this new gel application method.

Application of Coccidian Vaccine and the Evaluation of Field Scoring Methods

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Comprehension of administration strategies, their relative efficacies, and oocyst cycling patterns in vaccinated broiler flocks in the context of the scoring methods commonly employed for field monitoring are essential for successful vaccination programs. *E. maxima* was used as the marker organism to compare non-vaccinated, gavage, post-hatch aqueous spray and gel administered coccidiosis vaccine. The vaccine was administered at a commercial hatchery and mucosal count scores (MCS), histological count scores (HCS) and PCR were used to assess *E. maxima* cycling in birds placed into commercial houses. By characterizing *E. maxima* cycling on Days 6 and 17 using multiple methodologies with the objective to demonstrate any treatment differences as well as quantify the sensitivity of the methods used most commonly to assess infections during field monitoring of commercial flocks. Gavage and gel application were found superior to spray and non-vaccinated applications($p<0.05$). Field evaluation methodologies validated the use of MCS and HCS while the relative sensitivity of PCR for identification of *E.maxima* remains uncertain.

Rate of Ingestion/Uptake of *Eimeria* oocysts from Live Coccidia Vaccines via Different Application Methods of Inoculation

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Two live coccidia vaccines were compared via different delivery methods. The two different delivery methods were spray verses gel application. Commercial broiler chickens one day of age were vaccinated via one of three methods: spray, gel or gavage. Birds were later examined for uptake of *Eimeria* oocysts by intestinal scrapings, oocysts counts and histopathological examination. Under similar administration conditions, spray application of coccidian vaccines seems to provide a greater uptake when compared with gel application. Also, the number of available oocysts in the vaccine, irrespective of the delivery method or medium had the greatest impact on the percentage of positives.

Comparative Evaluation of Different Anticoccidial programs in Broilers with specific Attention to Avatec 20 (Lasalocid Sodium)

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Coccidiosis control is one of the most costly health management practices in poultry industry. This study aims to evaluate the use of several anticoccidial programs in broilers individually or in combination with other anticoccidial products. Avatec 20, a divalent

ionophore coccidiostats that contains 20 % of the active substance lasalocid sodium, was used in 5 different treatments in combination with several anticoccidial products, such as Nicarbazin, Deccox, and Maxiban. The design of the study included treatments, in the form of shuttle anticoccidial control programs and 4 dietary phases as Starter, Grower (days 17-25), Finisher and Withdrawal (days 37-41). Seeder birds were used to increase the level of coccidiosis challenge. Performance parameters of two consecutive broiler flocks raised to 42 days of age were evaluated. The $p \leq 0.05$ level of significance was used for all hypothesis testing with two sided tests. Multiple comparisons among treatments were made using the Shaffer simulated methodology. Mean body weights at 27 and 37 days of age for all treatments were significantly higher than non-medicated challenged control group. FCR for the challenged control group was also significantly higher than all other treatments for D27, D37 and D42.

Dynamics of *Eimeria* Oocyst Concentrations and Species Composition in Commercial Broiler Houses During Anticoccidial Drug or Vaccine Programs

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Effective control of avian coccidiosis depends on understanding the population dynamics of *Eimeria* during growout so that the most efficacious drugs/synthetics or vaccines can be chosen. In the present study, litter samples were collected at 0, 2, 4, and 7-8 weeks during growout over the course of 1.5 years from a total of 20-23 individual houses from 7 different commercial broiler farms, all from a single complex. *Eimeria* oocysts were recovered from these samples, counted by microscopy, and extracted for DNA followed by PCR for determining *Eimeria* species composition. During chemical/ionophore drug program, *Eimeria* oocysts were undetectable at 0 days, rose to about 2×10^4 oocysts/gram (oo/g) by 4 weeks, and decreased to 10^4 oo/g by 7-8 weeks growout. In a few farms, peak *Eimeria* oocysts numbers were observed later in growout (7-8 weeks), possibly reflecting drug-resistance in the *Eimeria* population. During vaccination program, initial oocysts concentrations at day 0 were about 10^4 oo/g, rose to 10^5 oo/g by 2 weeks, and then decreased to about 10^4 oo/g by 7-8 weeks. In general, *E. acervulina* and *E. maxima* were present at all timepoints irrespective of control program, with *E. tenella* and *E. praecox* regularly present at 2-4 weeks during growout. Of interest was the appearance of "minor" species such as *E. brunetti* during the anti-coccidial drug program, and *E. mitis* and *E. necatrix* randomly present during the vaccine program. These findings suggest that the particular control program (synthetics/drugs or vaccines) impacts the *Eimeria* population in litter on broiler farms.

Evaluation of the Efficacy of Different Coccidiosis Bioshuttle Programs and Dietary Capsicum-Turmeric Oleoresins on Broiler Performance

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Huvepharma, Inc.[1], Southern Poultry Research[2]

A total of 2400 one-day-old male broiler chicks were sprayed with a commercial coccidiosis vaccine and raised to 43 days of age in randomly assigned pens on built up litter. Feed conversion ratio and body weight were calculated on days 14, 35 and 43. Fecal samples from every treatment group were

collected, and oocyst counts were performed every 4 days from day 7 to the end of study. The treatment groups are as follows: Group 1 (G1): water soluble amprolium between days 14-16; Group 2 (G2): amprolium 72.6g/ton in feed between days 15-35; Group 3 (G3): amprolium 113g/ton in feed between days 15-35; Group 4 (G4): capsicum-turmeric oleoresins in feed from day 0 to day 43; Group 5 (G5): Salinomycin 50g/ton in feed between days 15-35; Group 6 (G6): no feed additive as a negative control.

There was no significant difference in adjusted feed conversion ratio or weight gain between groups at 14 days. At 35 and 42 days of age, G3 had the greatest weight gain and lowest adjusted feed conversion ratio, followed by G5 and G4. Short duration of water soluble amprolium 0.006% at days 14-16 was nearly as effective as continuous use of a low dose (72.6 g/ton) of amprolium from days 15-35. The capsicum-turmeric oleoresins combination product performed similarly to the amprolium and salinomycin treatments. The treatment group with amprolium (113g/ton) resulted in highest weight gain with the lowest feed conversion ratio.

Evaluation of performance of probiotics, amprolium, and a capsicum-turmeric phytonutrient in a necrotic enteritis challenge

Tina Yun-Ting Wang, Marilynn Finklin, Ben Johnson, TJ Gaydos

Huvepharma Inc

Three separate trials were conducted to evaluate the effects of potential antibiotic-free products (*Bacillus licheniformis (BL), Bacillus subtilis (BS)*, dietary capsicum-turmeric oleoresins (CT) or amprolium) to ameliorate necrotic enteritis (NE) and improve growth performance in broiler chickens. All birds each trial were sprayed with a full dose of coccidiosis vaccine and raised to 42 days (Trials 1 and 3) or 22 days (Trial 2). Different probiotics, natural products, or the feed additive amprolium were evaluated in the trials. Birds were challenged in Trials 2 and 3 with 10^8 *Clostridium perfringens (CL)* for 3 consecutive days starting at day 18. In addition, 20ml of *E. maxima* per pen was spread around feeders and drinkers at day 14 in Trial 3 to enhance coccidiosis challenge level. In Trials 2 and 3, birds were necropsied one day after the CL challenge to evaluate NE lesions. Body weight and feed conversion data were recorded in all Trials. BMD (50g/ton) was also evaluated for necrotic enteritis control in Trials 2 and 3. The results from Trial 1 showed that adjusted FCR at day 42 for *BL* and *BS* groups, with or without amprolium (72.6 g/ton) in the grower feed, were significantly lower than for the non-additive control group. The results from Trial 2 indicated that there was no significant difference between the treatment groups(*BL, BS* and CT product) and the BMD 50g/ton positive control group on mortality, body weight, NE lesion score and adjusted FCR during NE challenge conditions. The results form Trial 3 exhibited that *BL, BS* and the CT combination product had the lowest NE lesion scores at 21 days during the peak of the NE mortality, and significantly heavier weights in the period past the disease peak at day 35. At day 42, the combined product still had the lowest adjusted FCR, even lower than the non additive non challenge group. Overall, *Bacillus licheniformis, Bacillus subtilis* and the capsicum-turmeric oleoresin products, alone or in combination, were as or more effective than the antibiotic BMD in prevention of clinical NE, and equally important, the negative effect of subclinical on growth performance.

Effect of probiotics on Marek's disease vaccination

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There is growing interest in probiotic and fermentation products as alternatives to antibiotics as growth promoters, or to inhibit pathogens such as Salmonella. We tested two commercially available products for the purpose of evaluating potential interference with Marek's disease (MD) vaccines when administered *in ovo*, as well as to see if the products were beneficial to MD protection. The first product, from Company A, was a live lactic acid bacteria probiotic. The second product, from Company B, was a yeast (*Saccharomyces cerevisiae*) fermentation product. We conducted three trials with birds that were vaccinated with HVT, treated with probiotic or fermentation product (administered *in ovo* or in feed), and challenged with various vvMDV strains. The first two trials, using vvMDV strain 583A, resulted in low incidence of MD in HVT-vaccinated groups with and without both products, demonstrating there was no interference with the MD vaccine. The third trial, using vvMDV strains Md5 and 612, resulted in higher incidence of MD in all HVT-vaccinated groups. Across all three trials, MD incidence was slightly lower in vaccinated birds given the probiotic or fermentation products when challenged with the two lower virulent strains (583A or Md5), although differences were not statistically significant. Probiotic and fermentation products may have value in MD protection when faced with lower virulent MDV strains, however, additional studies are needed to validate the results and to better understand how these products affect the immune system.

Evaluation of Viral and Host Mechanisms Involved in Permanent Marek's Disease Virus Induced Immunosuppression

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Marek's disease virus (MDV) can produce severe immunosuppression (MDV-IS) in chickens by various mechanisms. We have recently developed a model to study MDV-IS that occur in commercial chickens bearing maternal antibodies against MDV and in the absence of lymphoid organ atrophy. In this model, MDV-IS is indirectly evaluated by assessing the ability of MDV to reduce the efficacy of infectious laryngotracheitis (ILT) vaccines. A series of experiments were conducted to (1) standardize the model; (2) evaluate the role of pathotype on MDV-IS; (3) evaluate efficacy of various MD vaccines against MDV-IS; and (4) to evaluate the role of tumors on MDV-IS. Our results showed that early infection with very virulent plus (vv+) but not virulent or very virulent MDVs jeopardized protection conferred by ILT vaccines. None of the currently used vaccine protocols, even those that were highly protected against MDV-induced tumors (HVT+CVI988 in ovo, or HVT in ovo followed by CVI988 at day of age) protected against MDV-IS. However, vaccination with experimental vaccine rMd5-

BACΔMeq at day of age resulted in total protection against both tumors and MDV-IS. The lack of correlation between MDV-IS and tumors was confirmed by a retrospective study using results from four animal experiments. Furthermore, comparison of MDV-IS induced by vv+ strain 686 and related viruses (r686-BAC and r686-BAC∆MEQ) reaffirmed that tumors could occur in absence of MDV-IS. A gradation of virulence and immunosuppressive abilities among 686, r686-BAC, and r686-BAC∆MEQ was observed and suggest that MDV-IS is a feature acquired by the most virulent MDV strains.

In ovo vaccination of commercial meat type chickens with herpesvirus of turkey: vaccine replication and effect on the chicken immune system

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Newly-hatched chicks get exposed to numerous antigens within the first few days of life when their immune system is nascent. In commercial chicken flocks, the highest mortality generally occurs during the first week. To avoid early mortality associated with infection, antibiotics are commonly administered in the hatchery. The use of antibiotics is now becoming restricted and so this might not be an option in the near future. Vaccination in ovo, now commonplace for Marek's disease (MD) is an alternative means for providing additional protection via innate and adaptive immune responses. Administration of herpesvirus of turkeys (HVT) to 17-19 day old embryos (E17-E19) has been shown to increase protection against MD. Furthermore, we have recently demonstrated that administration of HVT to 18 day old SPAFAS embryos hasten maturation of the chicken immune system. In our previous studies, 1-day-old SPAFAS chickens that received HVT in ovo had an expansion/activation of cell phenotypes in spleen at levels comparable to 1-2 weeks-old chickens. In addition, HVT vaccinated chickens responded better than sham-inoculated chickens against two unrelated antigens PHA-L and KLH. The objective of this study is to evaluate if various HVTs (conventional and recombinant) have a similar effect in commercial meat type chickens. Three experiments were conducted to evaluate the replication capability of various HVTs in the chicken embryo, the effect of various HVTs on the chicken embryo immune responses, and the effect of in ovo vaccination on the immune responses against non-related antigens. Results will be discussed.

Assessment of the Signaling Synergism of Bivalent HVT/SB1 Vaccine Components on Innate Sensing and Acquired Immune Patterning

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The purpose of this study is to determine the mechanisms of protective synergism conferred by SB1 upon bivalent vaccination. Using targeted transcriptome analysis, we analyzed the expression profiles of innate and acquired immune signaling genes in the spleen cell suspensions isolated from SPF chickens over a time period of 2, 6, 24, 48, 72 and 96 hrs post vaccine treatments (Diluent Only, HVT Only, SB-1 Only. HVT/SB1). Relative expression changes were based on comparison with diluent only control with the geometric mean of several cellular genes as a

reference. There was a significant induction (fold change > 2.0, $p<0.05$) of innate immune responsive genes (interferon signaling and pro-inflammatory markers) by 72 hrs post vaccine treatment. In addition, there was a synergistic activation of innate immune responsive genes in bivalent vaccine treatments when compared to HVT Only, SB-1 Only treatments. This provides a rational basis for the complementation afforded by the SB1 in the bivalent vaccine. Further studies will be focused on validation of these markers at the protein level and acquired immune patterning.

Co-infection with fowlpox virus strains carrying variable sequences of reticuloendotheliosis virus

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Fowlpox virus (FPV), the type species of the genus *Avipoxvirus*, is a large double-stranded DNA virus that replicates in the cytoplasm of infected cells. Sequences of the avian retrovirus, reticuloendotheliosis virus (REV) are frequently found within the genome of FPV strains. Notably, while vaccine strains of FPV usually carry remnants of the REV long terminal repeats (LTRs), field isolates have been shown to harbor either the REV LTRs or near the full-length REV provirus. Here we present evidence of natural occurrence of heterogeneous FPV populations carrying the REV LTR and the full length provirus in wild turkeys. Avipoxvirus-like disease was observed in a juvenile male Merriam's wild turkey in Bon Homme County, South Dakota. The disease was characterized by yellow papules distributed throughout the non-feathered areas of the head. Avipoxvirus DNA was detected in lesion samples by polymerase chain reaction (PCR) and APV-like virions were observed by transmission electron microscopy. High-throughput DNA sequencing performed on nucleic acid extracted from skin lesions revealed two FPV genome populations carrying either a 197-nt remnant of the REV LTR or a 7543-nt fragment corresponding to the full-length REV provirus. The complete genome sequences of these two populations were obtained. Notably, PCR testing of the sample above, a second clinical sample from a wild turkey and a FPV isolate obtained from chickens confirmed the natural occurrence of the heterogeneous FPV populations. Results here provide evidence of co-infection with heterogeneous FPV populations containing variable sequences of REV into their genome. These populations are likely to result from homologous recombination events that take place during FPV replication *in vivo.*

Nucleotide Sequence Analysis of Selected Genes of Avianpox Viruses

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Avianpox viruses have large size genome Although the nucleotide sequence of a vaccine like fowlpox virus genome has been determined; the functions of many of its genes have not been evaluated. In addition to variable pathogenicity, fowlpox virus strains show genetic and antigenic differences e.g. strains associated with outbreaks in vaccinated chicken flocks reveal insertion of full-length reticuloendotheliosis (REV) in their genome while vaccine strains lack full-length REV. Avianpox viruses that infect wild birds show significant antigenic, genetic and

biologic differences from fowlpox virus. We have selected specific primers sets from the genomic sequences of fowlpox virus that will amplify fragment of various sizes by polymerase chain reaction (PCR). Using gene specific primers from fowlpox virus genome we have amplified fragments of corresponding genes in other viruses e.g.; A-type inclusion body gene, which is conserved in most of the viruses that we have tested. In order to comparatively analyze the nucleotide sequences of specific genes of fowlpox virus strains as well as those that infect wild birds, our studies are in progress to sequencing 39 K gene and photolyase gene specific PCR amplified fragments from genomes of these viruses.

Outbreaks of acute respiratory fowlpox in layers in France

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During fall 2015, two independent cases of fowlpox were diagnosed in commercial layer farms in western France. These farms showed very good management and biosecurity. In farm #1, an increase in mortality was suddenly observed and reached as much as 2% daily mortality. Diseased birds showed severe dyspnea and suffocation before death. The main macroscopic lesions were a very severe necrotizing tracheitis. Almost no cutaneous lesion could be observed in any bird. Histopathological lesions on the trachea included necrosis, vacuolization and cytoplasmic inclusions, highly suggestive of poxvirus infection. In farm 2, the clinical picture was similar, though less severe. All tracheal swabs and tissues sampled in both farms tested positive for PCR targeting *p4b* gene shared by all avipoxviruses, as well as for fowlpoxvirus-specific *fpv 140* gene. Furthermore, reticuloendotheliosis (REV) insertions were identified in the genomes of both viruses. Phylogenetic analysis showed that the viruses detected in the 2 cases showed a very high genetic similarity, although no apparent epidemiological link could be identified. The viruses were isolated on chicken egg embryos and experimental infections through the aerosol route reproduced successfully the pathological picture.
Altogether these results illustrate that fowlpoxvirus may be the agent of very severe respiratory pathological picture. The specific pathological profile of this fowlpoxvirus needs further virological and pathobiological investigations.

Application of next-generation sequencing (NGS) in diagnostic avian virology

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Next generation sequencing (NGS) methodologies have revolutionized the field of genomics and have enabled significant contributions to multiples areas in virology, especially virus discovery and genomic characterization and pathogenesis. By using NGS in diagnostic avian virology, we have confirmed real-time RT-PCR false avian influenza virus (AIV) detection in waterfowl and swine swab samples, and detected naturally co-infections of both avian reovirus and adenovirus from one isolation and two

different variant strains of avian reovirus genotype 3 and 5 from one reovirus isolation. The NGS is a very powerful tool and has great potential in diagnostic virology.

Time as critical parameter for vaccination of Turkeys against Newcastle Disease

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Newcastle disease (ND) is considered to be one of the most important diseases of poultry worldwide, with vaccination successfully applied for disease protection. However, for turkeys experimental data on efficacy of vaccination is limited. This study was designed to test age and dose dependence of live Newcastle disease vaccine on vaccination efficacy after oral vaccination of turkeys.

Initial experiments proved that a vaccine dose between 10^5-10^7 EID50/ bird, applied on 21 day of live (dol), was effective in inducing a protection rate above 90 %. However, earlier vaccination of turkeys with maternal derived antibodies (MDA) failed to induce protection, despite replication of vaccine virus. Subsequent experiments, using a vaccine dose of 10^6 EID50/ bird, demonstrated that protection was mounted within 14 days: All animals that were exposed to challenge infection at the day of vaccination or 7 days post vaccination (dpv) suffered from disease indistinguishable to the non vaccinated control animals. Mortality and clinical disease stopped in vaccinated animals challenged 14 dvp. This protection lasted up to 8 weeks post vaccination (pv) and was accompanied by surcease of viral shedding. Vaccination induced antibodies were temporarily detectable by heamagglutination inhibition (HI)-test from day 14 pv until 6 weeks pv, i.e. late after vaccinated animals were HI-sero-negative but still protected.

These data support the notion that for MDA-positive turkeys, time of ND-vaccination is critical but already single oral immunization induces protection between the 5th and up to the 11th week of life and can limit spread of NDV.

Evaluation of in vivo replication of a Newcastle disease live attenuated vaccine strain in commercial broiler chicks: a comparison of drinking-water, spray and eye drop vaccination methods

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Newcastle disease virus (NDV) is one of the most important infectious agents in the poultry industry causing huge economic losses worldwide. Vaccination in endemic countries is widely used in order to keep NDV controlled. Several live attenuated vaccines are available on the market and the efficacy of these vaccines has to be tested in laboratory conditions in order to validate their use in the field. In the present studies a commercial live attenuated vaccine against ND (VG/VA AVINEW) was administered in commercial broiler chicks by spray, drinking water and eye-drop under controlled conditions. Target organs for vaccine virus replication were collected at different days post vaccination (DPV); the vaccine virus was recovered by RT-PCR. The detection of the NDV vaccine was compared between the different routes of administration at different ages. The NDV vaccine replication patterns were correlated with validation of vaccine virus tropism,

respiratory and digestive, whatever the route of administration. As already described in the literature for IB and aMPV live attenuated vaccines, replication patterns of eye drop or drinking waterl routes were slightly different from the spray.

Effect of live or live & inactivated Newcastle vaccination programs against virulent Newcastle Disease virus genotype VII challenge in controlled studies with SPF chicken or commercial layer – Clinical protection, viral shedding

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The protection of Newcastle Disease (ND) vaccination programs, single (eye-drop at day old) or repeated (eye-drop at day old + day 11 or 17) live vaccine VG/GA Avinew alone or in combination with inactivated vaccine (0.1 ml s.c. at day old or day 7), was assessed in SPF and commercial layer chicken against a recently emerged virulent NDV genotype VII Indonesian isolate (challenge: 5.0 log 10 EID50 NDV by intramuscular injection; observation period: 14 days post challenge). The SPF birds receiving two live ND vaccinations at days 1 + 11 showed a 95% clinical protection against virulent day 21 NDV challenge, whereas the groups with one or two live ND vaccinations plus inactivated ND vaccination at day old were 100% protected. The commercial layer groups receiving one (D1) or two (D1 + D17) live ND vaccinations showed clinical protection of 40% or 80% respectively against day 28 virulent NDV challenge. When commercial layer received additional inactivated ND vaccination at day 1 or day 7, the clinical protection against challenge increased to 90% or 100%. Assessing the shedding of the NDV challenge virus by qPCR on oro-pharyngeal swabs at 6 days post challenge, only in the groups receiving the additional inactivated ND vaccine at day 7 all birds remained negative and no virus shedding was detected. All not vaccinated but challenged SPF or layer birds died within 6 days. The results obtained confirm the efficacy of a live plus inactivated ND vaccination program against highly virulent genotype VII Indonesian NDV.

Productivity, clinical performance and seroconversion of broilers vaccinated with a vector *r*HVT-$F_{protein}$ Newcastle Disease (ND) vaccine and reared in non-vaccinating and velogenic ND virus (vNDV)- free poultry companies.

Luiz Sesti, Carlos Kneipp, Leandro Bianchet

Ceva Animal Health - Latin America

Several parameters of productivity, clinical performance and seroconversion for Newcastle were evaluated in commercial broilers vaccinated ad day one with a vector (*r*HVT-$F_{protein}$) Newcastle Disease (ND) vaccine in large field trials carried out in vertically integrated companies in southeast (Company 1) and southern (Company 2) regions of Brazil. Both companies had not vaccinated their broilers against ND for more than 20 years.

In Company 1, 943000 broilers were vaccinated with the vector ND vaccine at day 1 (SQ) and their performance compared to 902000 contemporary broilers. In company 2, 8 specific integrated producers (total around 320000 broilers) had their flocks vaccinated (*in ovo*) with the vector vaccines and their productivity, clinical parameters and serology compared with the immediate previous flock in the same farm.

At both companies, there was no statistically different production and clinical results; although a trend for lower total-mortality was observed in company 1 as well as a trend for lower condemnation rate at slaughter (secondary bacterial infection, airsacculitis) in company 2 for broilers vaccinated with the vector ND vaccine.

In company 1, seroconversion to Newcastle of broilers vaccinated with the vector ND vaccine was not different from that of broilers not vaccinated at all against ND. Notwithstanding, in company 2, Newcastle titers of broilers vaccinated with the vector ND vaccine were significantly lower ($p<0.05$) and more uniform ($p<0.05$) than those observed in previous flocks in the same farm. Therefore, the vector vaccine was quiet effective in protecting the flocks against the circulation of ND vaccine virus strain present in those farms (originated from nearby long living birds' farms, i.e., breeders and layers).

In conclusion, the use of a vector (*r*HVT-$F_{protein}$) Newcastle Disease (ND) vaccine in broilers reared in non-ND vaccinating and velogenic ND virus-free production systems will still allow monitoring of an eventual vaccine and/or field NDV infection through a simple serology at slaughter age. Therefore, offering the possibility of lower mortality losses, quick clinical diagnostic, and rapid and effective decision-making process towards NDV eradication in the affected production system.

Repeated challenge does not decrease the efficacy of NDV vaccines

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In the field, well-vaccinated birds may be repeatedly exposed to challenges with virulent strains of Newcastle disease virus (NDV), which are able to infect macrophages and cause damage to tissues of the immune system. In this study we evaluated the hypothesis that daily challenges with high doses of virulent NDV may overwhelm the immune system of well-vaccinated birds. Day old SPF white leghorns (n = 120) were vaccinated with a live NDV B1 vaccine [10^6 EID_{50} per bird, divided half intraocular (IO) and half intranasal (IN)], and two weeks later were administered a second NDV vaccine; either a live LaSota NDV (n = 60) using the same route and dose, or an inactivated LaSota oil emulsion vaccine (n = 60), given subcutaneously. One control group (n = 10) received one BHI inoculation (IO/IN) as a sham live vaccine and another control group (n = 10) received one oil emulsion with non-infected allantoic fluid as a sham inactivated vaccine. Thirteen days after the second vaccine serum was collected for all birds, and both control groups, and all of the NDV vaccinated birds (n = 120) were challenged with 10^6 EID_{50} of chicken/USA (CA)/2002 (CA/02) virulent NDV administered IO/IN. Half of the remaining birds vaccinated with two live NDV vaccines (n = 30) and half of the birds vaccinated with a live and an inactivated NDV vaccine (n = 30) were challenged with the same dose of CA/02 each day, for nine additional days. All sham-vaccinated birds died four days after

challenge. No morbidity or mortality was observed in any of the NDV-vaccinated birds until they were terminated 14 days after challenge.

Characterization of NDV Field Isolates and Evaluation of Protection against an NDV Isolate by a Recombinant HVT-ND and a Live Attenuated (C2) Vaccines in Commercial Broilers

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Newcastle Disease Virus (NDV) strains were isolated from vaccinated commercial broiler flocks exhibiting respiratory symptoms at the MS and TX complexes. Isolated NDV strains were characterized and compared with field and vaccine virus strains. One of the isolated strains was selected and used to evaluate the level of protection provided by a recombinant HVT-ND alone (in ovo) or in association with the live C2 vaccine (1 and/or 15 days of age). Vaccinated and control groups were challenged at 21 and 41 days of age with 10^4 EID_{50} per bird. The level of protection provided by different vaccination programs was evaluated based on clinical signs, serology, molecular detection, virus isolation and histopathology.

The use of Next generation sequencing in the diagnosis and typing of NDV isolates from commercial poultry flocks

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Newcastle disease (ND) is devastating for poultry production worldwide. NDV is capable of provoking grave disease in domestic fowl. Chickens infected with NDV show a wide spectrum of clinical signs that vary with different virus strains. Next-generation sequencing (NGS) has been widely applied to clinical diagnosis in humans and is progressively being used to monitor infectious diseases in animals. During ND outbreaks in vaccinated commercial poultry flocks in Pakistan, by using oropharyngeal and cloacal swabs, we have successfully detected and sequenced whole genome of NDV sub-genotype VIIi. Briefly, swab samples were amplified in 9-day-old specific-pathogen-free embryonated chicken eggs and further processed by next-generation sequencing. Total RNA from viral-infective allantoic fluid was extracted. Viral RNA was captured and enriched using SeraMega beads. Reverse transcription was performed using the Moloney Murine Leukemia Virus Reverse Transcriptase kit. The cDNA products were

purified, tagmented and amplified into Illumina libraries employing the Nextera XT DNA Library Preparation Kit. Fragment size distribution and concentration of the DNA libraries were checked on a Bioanalyzer 2100. Pair-end sequencing (2x250 base pairs) of the generated libraries was performed on an Illumina MiSeq instrument. Sequence data were assembled using MIRA software within a customized workflow on the Galaxy platform. The de novo assemblies were compared to GenBank databases and phylogenetic analyses were performed. The analyses showed that the detected NDV strains were most closely related to Pakistani NDV strains of sub-genotype VIIi. It is suggested that sub-genotype VIIi viruses were responsible for the outbreaks in vaccinated commercial poultry flocks.

National Poultry Improvement Plan

Denise Brinson

USDA-APHIS

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. Further, the NPIP establishes the regulatory standard in sample collection, diagnostic tests performed, and the laboratory protocols for conducting tests. Over the past year, modifications and changes to the NPIP federal regulations and the Program Standards document have been made. Lastly, there are several updates on activities performed within the NPIP.

The Commercial Duck Industry in the United States. Current disease challenges and contributions to the US Poultry Industry

Jaime Ruiz

Elanco Animal Health

The commercial duck industry in the United States is an important segment of the poultry industry. Traditional meat-type duck operations located in strategic areas of the country constantly supply high quality duck meat to specific markets. An historical review of the development of the duck industry will be reviewed. Past and present relevant disease challenges and current strategies for prevention and control will be discussed including the role of ducks in recent Avian Influenza outbreaks.

Tools for the Poultry Professional: How to Increase Personal and Professional Wellness

Andrea S. Zedek

Zedek Poultry Consulting, LLC

Many poultry veterinarians have demanding, stressful jobs. This may be compounded with compassion fatigue in certain situations, such as disease outbreaks requiring depopulation of infected flocks. Wellness of the veterinary professional is a relatively new concept, but one that is rapidly gaining recognition and research. This presentation will highlight initiatives by the AVMA Future Leaders Program from 2014-2016 to provide tools for veterinarians to increase personal wellness, as well as to guide the implementation of wellness programs in the workplace. Specific wellness activities geared toward the poultry professional will be presented that attendees

will be able to utilize immediately to increase their own personal wellness.

Emerging IBV variants: the genotypes, serotypes and pathotypes

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Department of Veterinary Medicine National Taiwan University

Infectious bronchitis virus (IBV), a member of *Coronaviridae* family, causes respiratory diseases in chickens, and poses economic threat to the poultry industry worldwide. Mass type strains have been applied as a control measure for IBV infection in Taiwan since early 1960s. However, since 2002, IBV variants that caused severe outbreaks have been isolated from the field. This study aims to characterize the genotype, serotype, and pathotype of IBV variants circulating in Taiwan. The phylogenetic analysis of the 3' structure protein gene region showed that these variants emerged through frequent recombination events among Taiwan strains, China strains, Japan strains and vaccine strains. Cross neutralization tests performed on the specific-pathogen-free (SPF) chicken embryos revealed that all four variants exhibited distinct serotypes. Clinicopathological assessment showed that all the variants possessed additional urogenital (kidney and oviduct) and digestive (proventriculus) tissue tropism, and two of the variants resulted in high fatality of 83% and 67% in one-day-old SPF chicks, respectively. Furthermore, the commercial IBV Mass type live-attenuated vaccine conferred poor protection against these variants. This study identified novel genotypes, serotypes, and pathotypes of emerging IBV variants circulating in Taiwan. There is an urgent need for an effective disease control.

Use of Infectious Bronchitis serotype specific probes for egg layers and broiler breeders

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In an effort to identify the circulating serotypes of infectious bronchitis in GA egg layers and broiler breeders from GA and other regions, spent chickens were sampled for IBV molecular detection and characterization. Serotype-specific probes for IBV developed at the Poultry Diagnostic and Research Center, University of Georgia (Mass, Conn, Ark, GA98, GA07, GA08, and GA13), were used to test samples obtained from spent fowl. Non-commercial poultry samples, as well as some samples previously confirmed as positive through virus isolation and sequencing from multiple sources were also tested. The results from this study will be presented.

Beyond diagnosis: Molecular epidemiological analysis of Infectious Bronchitis Virus

Mark W. Jackwood, Deborah Hilt, Sunny Cheng and Brian J. Jordan

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Infectious bronchitis virus (IBV) outbreaks can result from the emergence of new virus types but more often are from known IBV types reemerging during the winter months. The potential reemergence of one or more specific

IBV types can affect decisions on which vaccines are used. Typically, the diagnosis and identification of IBV types in the field involves reverse transcriptase-polymerase chain reaction (RT-PCR) amplification and sequencing of the hypervariable region of the spike gene subunit 1 (S1). The sequence of the identified virus is then used to report a percent similarity to previously sequenced isolates, but to date a molecular epidemiological approach has not been used to follow currently circulating strains. In this study, we conducted phylogenetic network analysis on the full-length S1 sequence and used date-of-isolation information to determine the genetic trajectory and time-scaled spread of specific IBV types currently circulating in the USA.

Molecular characterization of infectious bronchitis viruses from broiler chicken farms in Peru

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From January to August 2015 were analyzed by real-time PCR test a total of 200 samples of trachea, cecal tonsils and/or kidneys from broilers with suspicious signs of infectious bronchitis. A total of 110 samples were positive to infectious bronchitis virus. The samples were from farms located in major areas of the country (Lima, Trujillo, Ica, Arequipa Puerto Maldonado and Iquitos). For further gene sequencing, were selected 18 positive samples. The hypervariable region of spike protein 1 (SP1) was amplified by RT-PCR and sequenced to study the genetic diversity between the IBV strains. Phylogenetic analysis of the 18 sequences obtained was compared with other IBV sequences of worldwide. This analysis revealed that all the samples had close relationship with Q1 infectious bronchitis strain (Ref_IBV_S1d_concensus101_102) with nucleotide homology from 93 to 98%. This study indicates that Q1 strain of Infectious bronchitis virus is circulating among poultry farms in Peru.

Role of spike S2 ectodomain in attachment and selection of ArkDPI IBV vaccine subpopulations in chickens

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Specific minor subpopulations of ArkDPI IBV vaccines, differing in spike protein amino acid sequence from the major vaccine virus population, are rapidly selected in chickens, and likely cause the tracheal damage and vaccine virus persistence associated with these vaccines. The IBV spike protein mediates attachment to cells, the very first step in IBV infection and replication. To test the hypothesis that more efficient binding of spike proteins of specific subpopulations of ArkDPI IBV vaccines to susceptible cells in chickens contributes to selection of these minor subpopulations in chickens, we compared binding to chicken tissues of recombinant spike proteins representing vaccine subpopulations. We also compared binding of S1 subunit alone with binding of recombinant proteins containing the S1 domain and S2 ectodomain. It is generally accepted that the spike protein S1 subunit is responsible for attachment to susceptible host cells, while S2 mediates viral envelope fusion with the host cell membrane. However, we found that the S2 domain contributes substantially to binding affinity to relevant

chicken tissues. For one of the vaccine subpopulations strongly selected in chickens, addition of the S2 ectodomain markedly increased the affinity of binding to multiple relevant tissues and was necessary for binding to lung and kidney tissues. The S2 ectodomain was required for detectable binding of recombinant spike protein representing a weakly selected vaccine subpopulation to most chicken tissues tested. Thus differences in S2 of ArkDPI vaccine subpopulations selected in chickens could contribute to selection of these subpopulations at the attachment step of viral infection.

Characterization of Kidney Cell-Adapted IBV ArkDPI Vaccine during Back-Passages in Embryonated Eggs

Haroldo Toro, Vicky van Santen, Cassandra Breedlove

Department of Pathobiology, College of Veterinary Medicine, Auburn University

We previously demonstrated that adaptation of an embryo-attenuated infectious bronchitis virus (IBV) Arkansas Delmarva Poultry Industry (ArkDPI)-derived vaccine to chicken embryo kidney (CEK) cells shifted the virus population towards homogeneity in spike (S) and non-structural protein (NSP) genes. Based on S gene sequencing, the changes of the predominant Ark population after CEK adaptation were not reverted after one back-passage in embryonating chicken eggs or after a passage in chickens. We have also demonstrated that 5-day-old chickens vaccinated with CEK-adapted Ark were protected against Ark virulent challenge compared to unvaccinated-challenged controls. The previously described CEK-adapted ArkDPI vaccine virus (CEK passage 7) was further back-passaged for four additional passages in SPF embryonated eggs. The spike (S1) gene sequence of IBV RNA obtained from allantoic fluids after each egg passage continued to show no reversion. During all egg passages the viral load, determined by quantitative RT-PCR in the allantoic fluids, maintained similar levels. Finally, the extracted viral RNA was submitted for next generation sequencing to include both structural and non-structural genes.

Evaluation of the changes in IBV subpopulation composition using Next Generation Sequencing

Ha-Jung Roh, Roxana Sanchez-Ingunza, Melissa Madsen, Sean Brimer, Corey White, John El-Attrache

CEVA biomune

Avian infectious bronchitis virus, one of the most prevalent avian pathogens, may exist as a mixture of heterogeneous viral population due to its error prone RNA dependent RNA polymerase and recombinant events during virus replication. One of the most studied examples of genetic diversity in IBV population is Ark DPI-derived attenuated vaccines. It has been previously shown that Ark-DPI commercial vaccines contain subpopulations that behave differently in replication and pathogenicity. Additionally it has been suggested that selected minor subpopulation in the vaccine can possibly attribute to the persistence of Ark DPI type vaccine in the field. To understand viral population, traditionally cloning methods followed by Sanger sequencing or direct Sanger sequencing was used and virus populations were estimated based on chromatogram peak heights. However, detection of lower frequency variants requires deeper sequencing. Next generation sequencing (NGS) allows higher sequencing depth with increased throughputs. In this study, we have used Ion Torrent PGM platform

to get a better idea on IBV subpopulation composition and the changes driven by the host immune pressure. IBV vaccine samples were used in this study to set up the subpopulation baseline. Tracheal swab samples were collected from IBV vaccinated birds at different post-vaccination time points. Vaccine samples and tracheal swabs were processed for whole genome RNA sequencing. After defining the sub-genomic region with subpopulation, targeted deep sequencing (TDS) was used to determine the frequency of the subpopulation and potential haplotypes at greater sampling and sequencing depth. This study would provide a method for routine viral subpopulation analysis and help to view population diversity and viral evolution in detail.

Major Histocompatibility Complex and Innate Immunity as Factors Providing Genetic Resistance to Infectious Bronchitis Virus

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We have previously demonstrated that MHC alleles B18 and B21 were more resistant to an IBV M41 challenge; we also demonstrated that part of the resistance was reflected as a delayed increase in viral load in these lines. Further IBV resistance testing has been carried out in our laboratory including additional MHC B haplotype lines. In addition, we have been investigating local humoral responses in tears and cell populations such as macrophages that might be related with resistance to IBV infection. Results from these experiments will be discussed.

Is Infectious Bronchitis vaccination with Mass and QX genotypes protective against Q1 strain? - A case report

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Infectious bronchitis virus (IBV) causes a chicken disease characterized by respiratory symptoms and great economic losses. Many different IBV genotypes are circulating worldwide and are characterized by limited cross-protection. In Northern Italy IBV QX is currently the dominant genotype circulating. Homologous vaccination coupled with Mass vaccine at day 1 has been introduced. A monitoring program was carried out in some vaccinated farms. Tracheal and cloacal swabs were collected at 5, 8, 12, 15, 19, 23, 29, 37, 43, 50 and 57 days from 10 birds, and blood samples were collected every week from 20 birds. The swabs were processed in pool and 2 real-time PCRs specific for the two vaccines were performed. An HI test was performed on blood samples for IBV QX, Mass and 793B antibody titration. Mild respiratory symptoms were observed in one farm at day 16 for 24h. No mortality was present and no treatment was required. At day 43 swollen head syndrome, mild respiratory signs and increase of mortality were reported. Samples collected in presence of clinical signs were processed also for Avian Metapneumovirus (aMPV) and for IBV genotyping. IBV Q1 genotype was detected at days 15 and 50 and aMPV was detected at day 43. In our study the vaccination with a Mass genotype associated with a QX seems to be effective for the control of IBV Q1 strain infections based on clinical signs and productive parameters. This evidence extends the validity of protectotype

concept also to the recently emerged IBV Q1 genotype.

Control of Severe Respiratory Problems Caused by Arkansas-Related Strains of the Infectious Bronchitis Virus Using a Combination of two Heterologous IB Vaccine Strains in Broilers in Mexico

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For several years, severe respiratory distress, manifesting itself in the form of an obstructive fibrinoid tracheobronchitis, had been reported in broiler flocks in several poultry-producing areas in Mexico, particularly in areas where there are distinct seasonality changes and the dry-season is when the most respiratory problems used to occur. However, in recent years these problems appeared in broiler flocks regardless of the time of the year, causing high mortality, reduced daily weight gain and high feed conversion rates. In a central Mexico group of farms, comprising over 1.2 million broilers, where all showed very high mortality and decreased production parameters, several strains of Arkansas-related viruses where detected. The objective of this work was to demonstrate that the use of two different live virus vaccines, antigenically unrelated among themselves, but which had previously shown their effectiveness in controlling Arkansas-related strains in vitro, could replicate such results in the field under problematic environmental conditions, such as high altitude, high stocking density and high market weight and late market age. As a result, while all previous flocks in all farms the average mortality was 20%, FCR was slightly over 2.00, daily weight gain was 53.9 g, the following cycle in the same farms the average mortality was 4.9%, FCR was 1.96 and daily weight gain was 54.3 g, showing that the use of the Protectotype concept devised several years ago can be successfully applied in broilers grown under difficult management circumstances.

Evaluating Infectious Bronchitis Virus Vaccination by Gel Administration

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Infectious bronchitis virus (IBV) is a highly contagious, economically significant upper respiratory pathogen of commercial poultry. IBV is endemic anywhere poultry are produced, making control of the virus one of the highest priorities for a poultry company. IBV control has traditionally been through vaccination with a live attenuated virus of the same serotype as the field strain. Since the vaccine must infect and replicate in the upper respiratory tract to induce proper immunity, it has been applied in aerosolized droplets by a spray cabinet in the hatchery on day of hatch. Recent research has shown that applying IBV vaccine in a large volume increases vaccination efficiency, but producers fear that “wetting” the chicks will chill them and hurt performance. A new gel application technology has the potential to deliver a large volume of vaccine suspension without wetting the chicks and this technology has been employed by several poultry companies for coccidia vaccination. In addition, recent data has shown that IBV vaccines can be combined with coccidia vaccines to further increase

efficiency of the vaccination process. Thus, the purpose of this research was to evaluate the efficiency of vaccination when IBV vaccine is applied by gel rather than spray. Vaccine virus replication levels as well as replication location was measured and protection from challenge was evaluated. This data will be valuable for commercial poultry companies in designing the best and most efficient protocol for vaccination against IBV.

Session B

A Microfluidic Device Integrated with an Advanced Nanostructured Material for Rapid Avian Influenza Virus Capture and Detection

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Rapid virus detection is highly desirable in infectious disease control, especially for highly pathogenic avian influenza. In this project, we present a handheld (0.5cm×2cm) disposable device that is capable of rapidly detecting avian influenza virus (AIV) from poultry swabs. This microfabricated device captures AIV from a swab sample by employing physical size-based exclusion and identifies the isolated virus using on-chip indirect fluorescent antibody (IFA) staining and next generation sequencing (NGS). The device consists of patterned carbon nanotubes (CNTs) arrays and a microfluidics channel made of polydimethylsiloxane (PDMS), which is an FDA approved biocompatible polymer. Virus particles with diameter similar to CNT inter-tubular distance are captured inside the forest. CNTs are selectively synthesized on transparent glass substrate and are functionalized with nitrogen dopants to improve biocompatibility via a chemical vapor deposition. First, we characterized size-based capture performance by measuring the capture efficiency using different diameters of fluorescence labeled particles. Second, we constructed experimental swab samples by spiking H5N2 virus into a chicken tracheal swab collected from a healthy (virus-free) chicken. By applying on-chip IFA test using AIV H5 monoclonal antibody, strong fluorescent signals were detected at the CNTs porous structures. By performing on-chip RNA extraction, we demonstrated the AIV incubated in the chicken embryo with ~10^7 PFU/ml in concentration can be captured with ~90% efficiency, as measured by polymer chain reaction (PCR). Furthermore, we developed bioinformatics pipeline for whole genomic sequencing by NGS. The results show complete 8 segments of H5N2 sequences are assembled in completed length with a 10^3 coverage.

Transmission of Recent H5 Highly Pathogenic Avian Influenza Viruses in Chickens

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The recent outbreak of highly pathogenic avian influenza in the United States resulted in detection of virus in over 21 states with almost 50 million birds affected. The spread of the virus from wild birds to poultry was largely responsible for the wide distribution of the virus. Farm to farm spread in gallinaceous poultry appeared to be more important as the outbreak continued. The earliest isolates had

a high chicken infectious dose 50 (CID50) and did not transmit efficiently in chickens in a direct contact transmission model. Viruses isolated later during the outbreak from layer and turkey farms had a lower CID50 suggesting adaptation of the virus to chickens. However, if the virus resulted in infection, most or all the chickens died, demonstrating the lethal dose 50 and infectious dose 50 in chickens were similar. In contrast the virus in mallard ducks was able to both easily infect and transmit in contact control ducks. However, mallard ducks did not show clinical disease. Using reverse genetics technology, the hemagglutinin gene was compared from the early viruses to the later viruses to determine the role of this gene in infection and transmission of the virus in chickens. Viruses were made that had different hemagglutinin genes, but the same internal gene. These viruses were used in challenge studies to examine for infectious titer and transmission. The understanding of the genes important for transmission will provide better markers to predict viral biology.

Characterization of H9N2 Low Pathogenic Avian Influenza Viruses from Pakistan (2012-2015)

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The currently circulating Eurasian H9N2 low pathogenic avian influenza virus (LPAIV) has rapidly spread to become the most prevalent LPAIV in domestic poultry and resulted in significant economic losses from deaths and decreased egg production. The H9N2 LPAI viruses have been endemic in Pakistani poultry since 1998, but no new viruses have been reported since 2010. Here we report on new H9N2 LPAIV, three from 2015 and one from 2012, isolated from Pakistan and genetically characterized. All eight segments of the viruses sequenced in this study, 26A/2012, 10A/2015, 13A/2015, and 25A/2015 belonged to genetic group B. Sequence comparison showed that the 2009-2010 Pakistan H9N2 viruses and all of the viruses sequenced in this study had an identical amino acid residues L at position 226 in the HA RBS, suggesting that Pakistan H9N2 viruses are still potentially infectious for humans. Considering the history of gene reassortment with HPAIV and the presence of what appear to be mammalian host-specific markers, continued active surveillance in poultry and mammals is needed to monitor the spread and understand the potential for zoonotic infection.

The Multigenic Nature of the Differences in Pathogenicity of H5N1 Highly Pathogenic Avian Influenza Viruses in Domestic Ducks

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The Eurasian H5N1 highly pathogenic avian influenza (HPAI) viruses have evolved into many genetic lineages. The divergent strains

that have arisen express distinct pathobiological features and increased virulence for many bird species including domestic waterfowl. The pathogenicity of H5N1 HPAI viruses in domestic ducks varies depending on the virus strain; however, the viral factors contributing to these differences in pathogenicity are not well understood. We determined the pathogenicity of more than 30 H5N1 HPAI viruses in Pekin ducks and conducted full genome sequence comparisons of the viruses but failed to find common virulence markers. In order to determine which viral genes and specific changes contribute to the virulence of H5N1 HPAI viruses in ducks, we also used reverse genetics to generate single-gene reassortant viruses with genes from viruses differing in virulence. Exchange of the hemagglutinin (HA) or neuraminidase (NA) viral genes of A/duck/Vietnam/201/05 (virulent virus) (HA clade 2.4) in the A/chicken/Indonesia/7/03 (non-virulent virus) (HA clade 1) background resulted in increased mortality in ducks. However, different results were obtained with reassortant viruses generated from two H5N1 HPAI viruses from Egypt (both HA clade 2.3.1), also exhibiting different virulence in ducks, where more than one gene was involved in increased virulence. In conclusion, the factors influencing virulence of H5N1 HPAI viruses in ducks appear to be multigenic and cannot be attributed to one specific gene or genetic change.

Characterization of Avian Influenza and Newcastle Disease Viruses from Poultry in Libya

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On March 2013, the Libyan poultry industry faced severe outbreaks due to mixed infections of APMV-1 (Newcastle disease) and low pathogenic avian influenza (AI) of the H9N2 subtype which were causing high mortality and great economic losses. APMV-1 and H9N2 were isolated and characterized. Genetic sequencing of the APMV-1/chicken/Libya/13VIR/7225-1/2013 isolate revealed the presence of a velogenic APMV-1 belonging to lineage 5 (GRRRQKR*F Lin.5) or genotype VII in class II, according to the nomenclature in use. Three AI viruses of the H9N2 subtype, namely A/avian/Libya/13VIR7225-2/2013, A/avian/Libya/13VIR7225-3/2013, and A/avian/Libya/13VIR7225-5/2013, were isolated and found to belong to the G1 lineage. Analysis of amino acid sequences showed that the analyzed H9N2 viruses contained the amino acid Leu at position 226 (H3 numbering) at the receptor binding site of the HA, responsible for human virus-like receptor specificity. On March 2014, an outbreak of highly pathogenic avian influenza (HPAI) virus of the H5N1 subtype was diagnosed in a

backyard poultry farm in an eastern region of Libya. The H5N1 isolate (A/chicken/Libya/14VIR2749-16/2014) was detected by real time RT-PCR (rRTPCR). Genetic characterization of the HA gene revealed that the identified subtype was highly pathogenic, belonged to the 2.2.1 lineage, and clustered with recent Egyptian viruses. This study revealed the presence of a velogenic APMV-1 genotype and of two influenza subtypes, namely HPAI H5N1 and H9N2, which are of major interest for public and animal health. Considering these findings, more investigations must be undertaken to establish and implement adequate influenza surveillance programs; this would allow better study of the epidemiology of APMV-1 genotype VII in Libya and evaluation of the current vaccination strategies.

Correlation between Interferon Response and Protective Efficacy of NS1-Truncated Mutants as Influenza Vaccine Candidates in Chickens

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In development of live attenuated influenza vaccine (LAIV), nonstructural 1 protein (NS1 protein) can be targeted to reduce viral virulence and to enhance host immune response at the same time. However, the mutants encoding C-terminally truncated NS1 proteins (*NS1*-truncated mutants) differ in terms of their immunogenicity and protective efficacy. In our previous study, NS1-truncated mutants derived from a same parent virus were tested in chickens as potential LAIV, and only two of the mutants showed good protection against heterologous challenge. A follow up study showed a good correlation between *in vitro* type I FIN response and *in vivo* vaccine efficacy. In this study, we evaluated the type I IFN and IFN-stimulated gene (ISGs) responses in chickens vaccinated with the four NS1-truncated mutants, and tested whether it can be directly correlated with the protection efficacy. Our data demonstrates the upregulation of IFN-stimulated genes and accelerated antibody induction by the NS1 truncated mutants, and their correlation with protective efficacy. Further, through oral administration of recombinant chicken IFN alpha in drinking water, we provide direct evidence that type I IFN can promote rapid induction of adaptive immune responses and protective efficacy of influenza vaccine. In near future, the in-depth analysis of ISGs response by NS1-truncated mutants will be conducted using high-throughput sequence analysis, which will provide detailed mechanism of the effect of type I IFN response on efficacy of NS1 truncated LAIV candidates and form a basis of broad-spectrum LAIV development.

Peptide Nanoparticle-based Vaccine Protects Chickens against High Pathogenicity Avian Influenza Virus

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Subunit vaccines are generally less immunogenic than whole organism vaccines. Approach to address this issue is to display repetitive antigen in a potent adjuvant formulation. Self-Assembling Protein Nanoparticle (SAPNs) technology is an excellent platform to achieve this goal for developing a universal Avian Influenza Virus (AIV) vaccine. Two conserved antigens (M2e and Helix C) are repetitively displaying on our SAPN surface. To generate self-adjuvanted SAPN, flagellin peptide was built into each SAPN. In this study, self-Adjuvanted M2e/HelC/F-SAPNs can stimulate TLR5 *in vitro* in a dose dependent manner. Specific Pathogen-Free Chickens vaccinated with M2e/HelC/F-SAPN induce significantly higher titers of antibodies than unadjuvanted one. Antibodies from chickens vaccinated with the self-adjuvant vaccine are significantly more neutralizing towards H5N2 *in vitro* than those from animals vaccinated by the unadjuvanted one. Importantly, anti-sera induced by the self-adjuvanted vaccine also neutralize heterozygous subtypes of AIVs *in vitro*. SPF chickens vaccinated with M2e/Hel C/F-SAPNs were protected from challenge with H5N2 high pathogenicity avian influenza virus. We have shown 63% and 35% survival rates in chickens vaccinated intramuscularly and orally, respectively when compare to the unvaccinated control group at 2 weeks post challenge. Our data together indicates our M2e/HelC/F-SAPNs could be a potential vaccine for AIV.

Development and Application of a Vaccination Planning Tool for Avian Influenza

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The World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) have advocated for a comprehensive approach for the prevention and control of highly pathogenic avian influenza (FAO, 2004; OIE, 2007). OIE and FAO have recommended that planning tools be developed to assist countries in developing vaccination policies and practices (OIE, 2007; OIE2006). A vaccination planning tool was developed in response to requests from Asian countries. The tool has a linear structure, which assesses capacity unique to each country based on ranking and scoring. The tool was piloted at the request of the governments of Bangladesh and Nepal and consists of eight (8) planning clusters, 37 planning elements,

and 303 referenced planning criteria applied through a consultative planning workshop. The tool is designed to create baseline estimates and initiate a more detailed and ongoing process to: i) systematically follow up and further evaluate important gaps that must be addressed; ii) initiate a long-term consultative process and technical inputs to address the gaps; and iii) monitor and evaluate implementation of vaccination policies and programs. Overall scores of both pilot countries were obtained semi-quantitatively and 86% of participants indicated that the objectives of the planning tool and workshop were achieved. The avian influenza vaccination planning tool provides a systematic approach for decision makers to develop their national vaccination program for HPAI as part of an overall strategy for the progressive reduction and control of endemic influenza viruses in poultry.

Inactivated Vaccine Protects Layer Hens from Clade 2.3.4.4 H5 High Pathogenicity Avian Influenza Virus

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During 2014-2015, the U.S. experienced an unprecedented outbreak of H5 highly pathogenic avian influenza (HPAI) virus in wild birds and poultry, especially turkeys and layers, with over 49 million birds died or culled, and some countries banned imports of U.S. poultry products. In case a new wave would strike the country, vaccination could be a potential tool for prevention of H5 HPAI outbreaks. A reverse genetic H5N1 low pathogenic avian influenza vaccine seed strain with the clade 2.3.4.4 hemagglutinin (HA) gene was tested in specific pathogen free adult White Leghorn layer hens, which were vaccinated once and challenged 3 weeks later, or vaccinated twice, three weeks apart, and challenged 6 months later. Challenge was a lethal stringent dose of North American clade 2.3.4.4 HPAI virus. All vaccinated hens were protected against clinical signs and mortality, while sham birds experienced 100% mortality. Single vaccination significantly reduced oral shedding titers and the number of birds shedding, which was associated with hemagglutination inhibiting antibodies. Double vaccination elicited good long-lasting immune responses since they lacked anamnestic reaction, conferred clinical protection, and reduced virus shedding when challenged 6 months later. These studies support the use of genetically related inactivated vaccine for prevention of a North American clade 2.3.4.4 HPAI virus in layers, in the event the U.S. needs to apply vaccination to control HPAI outbreaks.

Higher Quantity of H5N2 Clade 2.3.4.4 High Pathogenicity Avian Influenza Virus Required to Infect Broilers than Leghorns but No Difference in Age Susceptibility

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H5N8 and H5N2 high pathogenicity avian influenza viruses (HPAIV) have caused infection, disease and/or mortality in poultry and wild birds in the USA (2014-2015). During outbreaks in Minnesota, turkey and leghorn chicken farms were affected but broiler farms were not. The absence of affected broiler farms could be the result of failure to introduce HPAIV onto the farms and/or genetic

resistance of broilers to infection. In this study, we tested the ability of A/turkey/Minnesota/12582/2015 (H5N2) HPAIV (TK/MN) HPAIV to produce infection in 5-week-old, 8-week-old and adult broilers using three different intranasal doses of virus; i.e. 2log10, 4log10 and 6log10 mean embryo infectious doses (EID50). At the 6log10 dose, all 5-week-old, 8-week-old and adult chickens died, but none died at the 4log10 and 2log10 EID50 doses. The mean chicken lethal dose (CLD50) was 5log10 EID50 for all age groups. The mean death time (MDT) was 4.8 days for 5-week-old chickens, and 3.2 days for 8-week-old and adult chickens which were not significantly different between the three groups. All chickens infected with the virus, died. In previous studies with 4-week-old leghorn chickens, the TK/MN virus produced CLD50 = 3.6 EID50 and MDT = 2.0 days, suggesting broilers were slightly resistant to TK/MN HPAIV when compared to white leghorn chickens, but there is no difference in susceptibility between different ages of broilers. However, this apparent lower HPAIV susceptibility of broilers may not fully account for the lack of affected broiler farms in Minnesota, as 6log10 EID50 in experimental studies caused infection and death.

Network Modeling to Predict Avian Influenza Immunity Distribution in the Poultry Industry in Vietnam and Bangladesh

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A model combining value chain network analysis and immunity modeling previously designed by CIRAD (EVADOC) was applied to predict the distribution of immunity against avian influenza in poultry industries in Vietnam and Bangladesh. This model required the collection of information over a two-year period on the production dynamics and capacities along with the organization of the poultry production network in these two countries. The model estimated the vaccine coverage for each node of the network and vaccination scenario, the positive sero-conversion levels and the duration of sero-protection according to different vaccination programs against Avian Influenza commonly used in these countries. Spatial analysis and cost-benefit evaluation were performed to assess the spatial distribution of the immunity along with the efficiency of the different strategies in the study areas. Results of this epidemiological modeling study will be presented.

A Simulation Based Evaluation of the Time to Detect EA/NA H5N2 HPAI Virus Infection in Commercial Turkey Flocks under Various Active Surveillance Testing Protocols

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During the recent outbreak of highly pathogenic avian influenza in Minnesota, targeted active surveillance using real-time reverse transcriptase polymerase chain reaction testing in dead birds was used to reduce the risk of HPAI virus spread through live bird movements. Time to HPAI virus detection within a flock of turkeys may vary considerably depending on the outbreak strain and active surveillance protocol used. Moreover, detection probability varies depending on when surveillance samples are taken relative to the time of movement. In the case of moving live birds, missed detections are a risk for further outbreak spread. We considered differences in transmission characteristics of the Eurasian /North American H5N2 HPAI virus isolated from wild birds in the first few weeks of the outbreak compared with H5N2 HPAI virus strains isolated much later, on model predicted time to HPAI virus detection in turkey flocks. We evaluated the performance of active surveillance sampling protocols where up to 5 or 11 oropharyngeal swab pools are taken from the daily mortality for every 50 dead birds from each house on two consecutive days prior to movement. As an exploratory analysis, we also compared time to detection via water sampling. However, there is considerable uncertainty in the predicted gain in time to detection due to the uncertainties in water sampling model inputs. We also evaluated active surveillance protocols where both oropharyngeal and cloacal samples are taken from the same dead bird (swabs are handled and transported in separate tubes) as an additional option to increase detection probability.

The BC Emergency Laboratory Database System (ELDS): Coordinated Management of Notifiable Avian Influenza Outbreak Information

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Four multi-agency containment responses to the detection of Notifiable Avian Influenza (NAI) in commercial poultry in British Columbia has clearly revealed an urgent need for a shared database to manage the information requirements surrounding the laboratory surge capacity in surveillance testing and the timely & efficient results reporting to decision-makers. Any delay in performing tests or results reporting can have a significant impact on the effectiveness and efficiency of disease containment measures.

Recognizing the specific jurisdictional roles & responsibilities of the provincial and federal government in responding to the detection of NAI, the Emergency Laboratory Databases System (ELDS) is designed to:

- Run independently from the current Vet Lab diagnostic reporting system,
- Have tightly controlled access security,
- Handle a high volume submission rate with rapid turnaround time,
- Document and track lab sample testing activity & disposition,
- Provide efficient and timely web-based reporting of results to pre-defined stakeholders,
- Capture premises-specific contact information & animal data that can be web-accessed to allow real-time containment activity planning, tracking and decision-making for that premise,
- Collate test results from multiple networked laboratories providing diagnostic support to the outbreak,
- Have the ability to generate a variety of summary reports that will facilitate reporting to multiple stakeholders,
- Provide a closed dataset of complete information captured over the duration of an outbreak for shared or independent analysis and epidemiologic review.

The BC ELDS addresses a serious and underappreciated vulnerability in collaborative disease outbreak management and will allow for more insightful and objective retrospective analysis of FAD events.

Pathogenicity of 2015 North American H5N2 Highly Pathogenic Avian Influenza Poultry Isolates in Chickens and Mallards

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Infectivity, transmissibility, and pathogenicity of four H5N2 highly pathogenic avian influenza (HPAI) viruses (clade 2.3.4.4) isolated from the Midwest poultry outbreaks in 2015 was studied in chickens and mallards and results compared to the results obtained with the index H5N2 virus (A/Northern pintail/Washington/2014). The mean death time in chickens infected with the poultry H5N2 isolates was earlier (2-3 days post inoculation) than the observed with the index H5N2 virus (3-7 days). Three of the poultry H5N2 isolates required a lower dose of the virus to infect the birds than the index virus. The fourth isolate had a similar mean bird infectious dose than the index virus, indicating that it was most likely a wild bird introduction. The viruses transmitted poorly to contact exposed chickens. In mallards, one of these poultry isolates was highly infective, and caused disease in some ducks, but the second virus appeared less adapted to ducks. However, all direct contact ducks became infected, with the exception of two ducks from the group inoculated with the low dose of A/Ck/IA/13388/2015, demonstrating the easy transmission of these H5N2 viruses among ducks. These results suggest that the more recent H5N2 HPAI viruses have increased infectivity and transmissibility for chickens

compared to the earlier H5N2 virus, indicating adaptation after circulating in poultry.

Investigation on the Possible Application of a Serological DIVA Monitoring Strategy when a rHVT-H5 Vaccine is Used to Control Avian Influenza.

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Vaccination against Avian Influenza (AI) is a tool that can be used in Poultry as a complement of an eradication program or more systematically for the control of the disease. In both cases, it is necessary to monitor the spreading of the field infection allowing detection of infected flocks under the cover of a vaccine protection.

Several experiments have demonstrated the capacity of a rHVT-H5 vaccine to induce protection against various subtypes and clades of H5 HPAIV, in the presence or not of specific maternally derived antibodies. This protection includes the elimination of clinical signs and reduction of shedding of the challenge virus, as well as an increased resistance to infection.

Because the vaccine does not contain any live or killed AIV, it was expected that a DIVA strategy based on serological testing could be implemented including detection of the vaccine virus replication and assessment of well vaccinated chickens.

In order to investigate this, several studies including challenge experiments have been conducted using various serological and molecular assays on a large number of chickens and for a long period of time after challenge allowing a better characterization of the humoral immune response induced by vaccination only or vaccination and challenge.

Heterosubtypic Immunity to Low Pathogenic Avian Influenza viruses in Mallard Ducks

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Long-term and cumulative heterosubtypic immunity in Mallard ducks challenged with different subtypes of wild bird-origin Low Pathogenic Avian Influenza Viruses (LPAVs) at different time points was evaluated. Fifteen 1-month-old Mallards were pre-inoculated with H3N8 LPAIV via choanal cleft and randomly divided into 3 challenge groups with 5 ducks each. Group 1 was sequentially challenged with H4N6, H10N7 and H14N5 LPAIV at 1-month intervals. Group 2 was challenged with H10N7 after 2 months post H3N8 LPAIV infection, while group 3 was challenged with H14N5 after 3 months. Single infection and age-matched control ducks were included for all challenges. We measured the duration and amount of viral shedding by virus isolation and RT-PCR in oral and cloacal swabs in different

time points and until day 14 post challenges. The development of cross-reactive humoral immunity was assessed by ELISA and virus neutralization.

Pre-exposure to H3N8 reduced the duration and amount of viral shedding after challenge with H4N6 and H10N7 LPAIV. Furthermore, H3N8 pre-exposure completely abrogated virus excretion of the HA and neuraminidase (NA) clade related H14N5 virus in ducks challenged with all 4 LPAIV. Serological results showed 13.3%, 20%, 40% and 60% of birds pre-challenged with H3N8, H4N6, H3N8-H4N6 and H3N4-H4N6-H10N7 respectively had detectable levels of cross-reactive neutralizing antibodies against H14N5. Our findings indicate that heterosubtypic immunity likely affect infection and transmission of LPAIV in Mallards and that the strength of immune responses might be influenced by the genetic relatedness of the HA and NA of the viruses encountered.

Preventing Outbreaks of Avian Influenza Through Timely Dissemination of Practical Science-Based Information

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The 2014-2015 HPAI outbreaks in the U.S. Midwest have re-emphasized an urgent need to develop, enhance, and disseminate practical, credible, science-based information on avian influenza prevention and preparedness, particularly biosecurity measures that specifically target the potential modes of spread or routes of transmission of HPAI virus. In order to be effective, biosecurity guidelines must be simple, easy to understand, readily accessible, and written in various languages spoken by poultry growers and workers. These educational efforts must be complemented by methods to ensure compliance with biosecurity measures on the farm and other points in the live production chain where AI virus can gain entry into poultry flocks. A key component of this USDA-NIFA Special Needs project at the University of Maryland is the production and distribution of short, high-impact biosecurity videos for commercial poultry growers, technical service personnel, and backyard flock owners. Highlights of these videos will be presented.

Inactivation of Avian Influenza Virus in Chicken Feed

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Highly pathogenic (HP) avian influenza (AI) viruses (AIV) belonging to the H5 or H7 types continue to threaten the world poultry industry. AIVs are relatively sensitive in the environment. However, AIVs are protected in the presence of organic material which increases resistance to physical and chemical inactivation. A more recent concern in AIV dissemination is the potential role played by chicken feed ingredients which could become contaminated from AIV positive wild birds via their feces. In this work we evaluated survival of AIV in chicken feed and determined the effectiveness of a commercially available disinfectant (Termin-8) at inactivating AIV in chicken feed. AIV was applied once at a dose of 10^5 EID_{50} per 1 gram of feed. Samples obtained at 1, 4, 16, and 24 hours showed that Termin-8 had quickly inactivated the virus at 1

hour compared to untreated controls. However, we also determined that AIV survival in untreated feed maintained at room temperature is relatively short (4 hours). Thus, to resemble natural conditions, in a second experiment instead of clarified virus, we added skimmed milk to the virus suspension at a ratio of 0.002% before contamination of the feed and evaluated AIV survival at room temperature (22°C). Under these conditions AIV survival in untreated feed increased to 24 hours post-contamination.

Inactivation of Avian Influenza Virus in Chicken Litter as a Potential Method to Decontaminate Poultry Houses

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Full cleaning and disinfection of a poultry house after an avian influenza virus (AIV) outbreak is expensive and labor intensive. An alternative to full house cleaning and disinfection is to inactivate the virus with high temperatures within the house. Litter in the house normally has a high virus load and is a difficult material to decontaminate, therefore litter was used as a matrix to evaluate the inactivation profile of AIV. Inactivation profiles were evaluated at 50°F-120°F at 10° intervals. Vials containing 1.5-2.0g of dry or wet litter were inoculated with 0.1ml of a recombinant H5N1 low pathogenic (LP) AIV with the HA protein of A/gyrfalcon/WA/41088/14 (with a LP cleavage site) ($10^{7.4}$ 50% egg infectious dose per vial). The vials were then placed in 1L containers composed of the same litter as the litter in the vials. Litter temperature and moisture levels were monitored for each temperature evaluated. Samples were taken at regular intervals for each temperature. Virus was extracted from each sample, and titrated in embryonated chicken eggs to determine the rate of inactivation. RNA was extracted from each sample for rRT-PCR analysis to determine the rate of viral RNA decay. The results from this experiment can be used as a guide for the length of incubation time required, at a specific temperature that will inactivate AIV inside of a poultry house.

Mutation of Avian Encephalomyelitis Virus during Embryo Adaption

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In the last years, outbreaks of Avian Encephalomyelitis (AE) following vaccination against AE have been described in California pullet flocks of 11 to 14 weeks of age. A part of the VP2 gene of the causative viruses from three cases, of the AE vaccine virus that had been used in one of the flocks as well as of the AE vaccine virus from a different company was sequenced. Comparison showed a high homology of more than 99% to each other and to published sequences from South Korea and Saudi Arabia. In contrast, homology to the embryo adapted van Roekel strain was only about 95%.

The field virus of one of these cases as well as the two vaccine viruses were isolated in embryonated specific pathogen free (SPF) chicken eggs. The infected embryos did not show lesions characteristic for embryo adapted AE virus strains like muscular dystrophy and immobilization of skeletal

muscles. It took several serial passages for the viruses to become adapted to the embryos. The mutations during the passages were monitored by next generation sequencing. The results will be presented and discussed.

Virological and molecular characterization of chicken astrovirus associated with reduction in hatchability, poor chick quality and "white chick disease".

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Liver samples from embryos and chicks were received for virological studies. These samples were obtained from flocks experiencing moderate to severe reduction in hatchability, embryonary mortality during the second week of incubation, and the presence of poor quality chicks "white chicks". Tissue samples were inoculated in SPF chicken embryonated eggs and chicken embryo liver cell cultures. Five to six days after inoculation, chicken embryos suffered mortality. The embryonic lesions included severe congestion, hemorrhages, and edema of abdominal muscles. Same mortality and lesion patterns were observed in two consecutive passages. No isolation of other virus was achieved. The presence of a chicken astrovirus was detected by RT-PCR, and the amplified product shared a similarity that ranged from 92% to 95% with the chicken astrovirus isolates from Georgia in the RNA-dependent RNA polymerase gene. A sequence analysis of other important genes will be included.

White Chick Syndrome in Ontario: Clinical features, Pathology and Viral Etiology

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White Chick Syndrome (WSC) has been recognized sporadically in Ontario broiler hatcheries for close to 30 years. In recent years, this syndrome has been more frequently observed, stimulating renewed interest in identifying the etiology which is now thought to be a chicken astrovirus based on results of recent European challenge studies. Clinical features, pathology and results of viral genotyping will be described.

Determination of baseline for bursa:bodyweight ratio in broilers in relation to sex & breed over age by using HTSi data package.

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Abstract: Evaluation of immune status of the flock is practiced across the industry while conducting the health surveys. In today's disease scenario, assessing and maintain good immune health is quite imminent and challenging. Avian Immune system consists of Organs like Bursa of Fabricius, Thymus, spleen, Bone marrow, gut associated lymphoid tissues, Harderian gland, Lymph nodes, cecal tonsils, payers patch, mackel's

diverticulum. Each of the individual organs has its prime role in maintaining good immune health. Though all the organs are important, Bursa of Fabricius stands as a leader in the immune system as it helps in humoral response by way of antibody production to the field challenge or vaccines.

There are many ways to assess the functioning of Bursa viz., Bursameter to measure the size, Bursal damage scoring, Bursal atrophy scoring, histopathology etc.,. All these parameters revel very vital information and useful in making good evaluation of bursal health. Another novel way of assessing the immune health could be through measuring bursa to body weight ratio. Though this concept is explained in the literatures, its practical application is very minimal. The bursa to body weight ratio vary based on age, breed, sex, management, stress etc.,. B-cells production would start from day 18 of the embryonic stage and goes until 3-4 weeks of age. Higher ratios would indicate better immune heath status provided the bursa is physiologically active. In this study, we have evaluated about 150 broilers at different age. The birds were weighted and slaughtered by using AVMA approved method. The B:B ratio has been defined based on breed and sex. The data was statistically analyzed for significance.

Evaluation of Efficacy of a live HVT-vectored IBD vaccine in a controlled study with SPF chicken against virulent challenges with classic IBDV, vvIBV or IBDV variant E type – Clinical protection, bursa health, viral shedding

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Classic virulent IBDV, vvIBDV and Variant E type IBDV are globally the most frequently isolated virus from Gumboro Disease field cases. In a controlled study, the protective effect of a live HVT-vectored IBD vaccine, injected in day old SPF chicks, was evaluated against virulent challenges by this three type of IBDV at 21 days of age. In contrast to positive control or sham vaccinated birds, the groups vaccinated at day old with the live HVT-vectored IBD vaccine showed full protection (no morbidity & no mortality, no visible bursa lesions) against virulent ocular challenges (2.0 log10 EID50 / bird) with either classic virulent IBDV Winterfield 2512 or vvIBDV Philippine isolate or Variant E type IBDV Philippine isolate. The bursa-body weight ratio remained unaffected at 5 and 10 days after each challenge in the HVT-vectored IBD vaccine vaccinated groups, but declined in the sham or not vaccinated and challenged control groups. Presence of challenge virus was assessed in cloacal and bursal samples by IBDV qPCR at days 5 and 10 post challenge. The individual bursa samples of all HVT-vector IBD vaccinated birds remained negative for all type of IBD challenge virus in contrast to those from sham vaccinated or positive control birds. No challenge IBDV was detectable in the cloacal samples of any group. The design of the study and the results obtained will be presented.

A Retrospective Study of IBDV Surveys Comparing Infection Dynamics Using Different Broiler Vaccination Strategies

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Early infectious bursal disease virus (IBDV) infections are most damaging to U.S. broilers because of their immunosuppressive potential—especially from early infections. However, even infections after three weeks of age can exact a performance cost. Vaccines administered to broilers can potentially complement maternal antibodies and serve as a buffer or "cushion" against the effects of early or late infections. Reductions in field virus shedding may also favorably impact future flock placements. There are generally 5 strategies for protecting broilers against IBDV: 1) no vaccine (simply rely on passive maternal immunity), 2) field vaccinate with an intermediate live vaccine or vaccinate in the hatchery with either 3) mild live vaccine, 4) HVT-IBD vector or 5) immune-complexed (I-C) live vaccine. Several field trials were conducted comparing I-C vaccine to one or more other strategies. Histological and PCR analyses were used to gauge actively infected flocks at different ages. In summary, while field infection windows varied between trial sites (presumably based on challenge pressure and maternal immune status), I-C vaccinated flocks tended to be infected less often than live or non-vaccinated flocks and for a shorter period of time compared to HVT-IBD flocks. Results, including quantitative PCR, will be shared and discussed.

Attempts to Determine the Source of *Escherichia coli* in Day-old Turkey Poults

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Escherichia coli can cause the disease colibacillosis, which can manifest itself in turkey poults as coliform omphalitis, yolk-sacculitis, or peritonitis. Poults are already colonized with and shedding high numbers of *E. coli* at placement. This study attempts to determine the source of these bacteria. Samples were taken from breeder hens, semen, poults and various sites in the hatchery, as well as brooder farms. The samples were streaked on Macconkey agar plates. Isolates were submitted for virulence factor determination and will be fingerprinted to determine if the isolates are similar. The information from this study may help focus attention on specific areas for prevention of colibacillosis in poults. The results are still being analyzed.

Avian Pathogenic *E. coli* in Hens and Day-of Hatch Broilers

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Avian colibacillosis is a systemic infection caused by *E. coli* and occurs most commonly in young broilers and poults. Avian pathogenic *E. coli* (APEC) comprise a specific subset of pathogenic *E. coli* that cause extraintestinal diseases of poultry. APEC consists mainly of enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) serovars. A

prerequisite for reducing APEC disease challenges in poultry is an understanding of the reservoirs for these organisms. In this study gastrointestinal tracts of 72 breeding hens and 80 day-of hatch (DOH) birds from the corresponding flocks from four complexes across three companies were examined for APEC. Results indicate that levels of APEC are inconsistent between individuals even within the same complex. The percentage of DOH birds with APEC levels above 1.0×10^4 CFU/g ranged from 5% to 80% demonstrating high levels of variation between complexes. The same can be said about hens as the percentage of birds with APEC levels above 1.0×10^4 CFU/g ranged from 0% to 72%. High levels of APEC in the hen did not necessarily dictate high levels of APEC in the DOH. Despite the sporadic levels of APEC, random amplified polymorphic PCR (RAPD-PCR) fingerprinting reveled a high level of relatedness between APEC strains isolated from hens and DOH birds suggesting that APEC strains are vertically transmitted from the hen to the chick. Focusing on controlling or reducing APEC populations in breeding hens will likely be an effective way to reduce the incidence of colibacillosis in broiler flocks.

Bacterial respiratory Diseases of Turkeys: Diagnosis and Control

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Respiratory diseases of turkeys are associated with severe economic losses, due to high mortality, high medication cost, drop in egg production in layer and breeder flocks and in many case low fertility and hatchability. In breeder flocks attention must be paid to prevent infections with vertically transmitted agents such as Mycoplasmas. Early recognition and monitoring programmes are essential in managing the infections and minimizing the economic impacts.

Several pathogens are incriminated as possible cause either alone (mono-causal) or in synergy with different other micro-organisms (multi-causal) or accompanied by non-infectious factors such as climatic conditions and management related problems. Worldwide the emerging and re-emerging respiratory diseases and or infections of turkeys are Avian Metapneumovirus (aMPV), Ornithobacterium rhinotracheale (ORT) and Fowl cholera (FC) infections. In addition, Avian Influenza (AI), Newcastle disease (ND) and Mycoplasma infections appear to cause problem in some countries.

The main ways to control any infectious disease are to prevent the introduction in an area supposed to be free from the infectious agent by regular biosecurity and monitoring and in case of the presence of the infection in one area measures should be taken to prevent the spread to other places. The following measures can be applied such as biosecurity, movement restriction, treatment, vaccination and in some cases eradication. The present paper it is not possible to review extensively the entire field of bacterial respiratory diseases, instead, this paper is limited to the treatment and control measures of some bacterial infections.

Genotypic and Phenotypic Assessment of *Gallibacterium anatis* isolated from poultry in the United States

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Gallibacterium anatis belongs to the family Pasteurellaceae and it is normally isolated from the upper respiratory tract and the

reproductive tract in chickens. The bacteria have been implicated in cases of peritonitis in poultry in the US and the infection is possibly associated with lesions in the reproductive tract leading to alterations in the egg shell. Frequent isolation of *Gallibacterium anatis* from septicemic cases causing mortality urges the need for interventions in these situations. The use of autogenous vaccines is an alternative for controlling *Gallibacterium* infection in chickens. The selection of the bacterial strains to be used in the vaccine formulation is complicated due to the fact of the high variability that may be observed between isolates from the genomic standpoint. We present a more comprehensive study of the phenotypic and genomic characteristics along with the evaluation of virulence of *Gallibacterium* in a chicken model, clinical history and pathological findings as a viable alternative to identify the most relevant strains for an autogenous vaccine preparation. Our approach also provides information on phenotypical characteristics and genomic details that aid in the understanding of the epidemiology of the disease in chickens.

Fatty liver disease and osteoporosis (cage layer fatigue): predisposing factors or consequence of *Salmonella* Gallinarum in commercial laying hens?

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Fowl typhoid (FT) caused by *Salmonella* Gallinarum (SG), is a severe disease mainly observed in brown egg layers. Previously, many predisposing–persistence factors have been evaluated in commercial flocks in Latin America. Two special conditions have been found in some SG positive flocks: fatty liver syndrome (FTS) and osteoporosis (cage fatigue). The affected flocks showed high feed intake and overweight previous to the presentation of FT. High prevalence of obese hens with abundant abdominal and subcutaneous fat with yellow and friable livers have been observed during the necropsy of birds that were suspected died as a consequence of FT. Other birds have shown severe deviation of the keel, bone fragility and enlarged parathyroid. Salmonella sp. was isolated from these birds and was characterized as S. Gallinarum by Intergenic Sequece Ribotyping. Other flocks of the same farm with the same conditions have suffered from severe cases of FT, with high mortality and severe egg drop production; while flocks without these conditions have showed milder FT signs or they have remained negative to SG.

Simultaneously to the management of FT, actions to improve the liver health were carried out, with special focus to the feeding program and the use of hepatic protectors. Calcium was supplemented according with the age and the level of egg production. These two measures have also been implemented in SG-negative flocks of the same farm. Prior to the presentation of an outbreak of SG, liver health plays a major role as predisposal factor of FT, it means that hens with pre-existing liver problems may be more susceptible to FT.

Modulating Escherichia coli virulence in various stages of production using organic acids and essential oils

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With fewer antimicrobials available, and reduction or regulation of these molecules taken out of the hands of those who are

closest to the production chain, integrators and producers are faced to adopt a new perspective. Early chick mortality linked with *Escherichia coli* can be associated with reducing or eliminating antibiotic use at the hatchery. An organic acid and essential oil blend was tested against other alternative products, such as fatty acid salts, probiotics or other essential oils, for the modulation of *Escherichia coli* virulence genes in the feces. Birds were divided in three treatments for the first trial and five treatments for the second trial. Birds received a blend of protected organic acids and essential oils for 14 days after hatch. Pooled feces were collected per treatment and submitted to the OIE Reference Laboratory for *Escherichia coli* (EcL Laboratory) in Québec, Canada. Virulence genes compatible with avian pathogenic E. coli were tested using multiplex polymerase chain reaction. Results favors the blend of organic acids and essential oils by reducing the number of samples or clones with virulence profiles of E. coli associated with APEC.

Microbial analysis of bioaerosols in poultry houses: a comparison of different poultry production types

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Air quality in poultry houses depends mainly on air temperature, air speed, relative humidity and presence of ammonia. Some other parameters may contribute to air quality, such as dust (load, size of particles) and presence of microorganisms (viruses, bacteria, fungi, archaea) alive or dead, including some of their products (endotoxins). Knowledge on the microbial quality of the air in poultry houses is still partial: references on microbial loads in poultry bioaerosols are still lacking.

We used an innovating cyclonic bioaerosol sampler to collect air samples in different systems of poultry houses: most of the French poultry production types were included in the survey: broiler chicken, free-range chicken, layers, turkeys, broiler Muscovy ducks and Guinea fowl.

We determined bacterial and fungal loads using both culture on agar plates and quantitative PCR targeting bacterial 16S, Archaea 16S RNA and *Aspergillus* spp.A. For each type of sample, a set of bacteria or fungi was typed using MALDI-TOF spectrometry. Factors associated with variations of loads and species profile were investigated.

This study provides a comprehensive picture, both quantitative and qualitative, of microbial quality of the air in poultry houses, in relation with the respiratory health of poultry and poultry workers.

A two-year retrospective study of bacterial enumerations from commercial broiler breeder fecal samples

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An analysis of bacterial levels of important chicken intestinal microflora populations was conducted from fecal samples of commercial broiler breeders. Fecal samples were collected over a two year period; July 1, 2013 to June 30, 2015. Bacterial enumeration analysis using culture media techniques was conducted on the fresh samples for levels of Enterobacteriaceae, *Salmonella spp*.,

Clostridium perfringens, Enterococcus spp., Staphylococcus spp., and *Lactobacillus spp*. Multiple representative fecal samples were collected from each commercial pullet and broiler breeder chicken farm for statistical analysis. Bird age ranged from 7 to 65 weeks-of-age for pullets and broiler breeders. Average enumerations of Enterobacteriaceae ranged from 8.40 $\log_{10}$ in a flock of 22 week-old chickens to 6.40 $\log_{10}$ in a flock of 65 week-old chickens. A significant negative linear regression for all age samplings was associated with Enterobacteriaceae enumerations (P= 0.023; decreased with age). A range of average enumerations for *Staphylococcus spp.* was from 5.97 $\log_{10}$ in a flock of 52 week-old chickens to 7.92 $\log_{10}$ in a flock of 18 week-old chickens. Enumerations of *Enterococcus spp.* ranged from 5.96 $\log_{10}$ in a flock of 55 week-old chickens to 7.76 $\log_{10}$ in a flock of 10 week-old chickens. A significant linear regression with age was associated with *Enterococcus spp*. enumerations (P=0.016; increased with age) in pullets, but not in breeders (P=0.851). A similar analysis of *Staphylococcus spp.* enumerations also showed a linear regression with age (P=0.059; decreased with age) in pullets, but not in breeders (P=0.614). The family Enterobacteriaceae are generally considered representative of pathogenic and non-pathogenic enteric genera such as *Escherichia* and *Salmonella*. *Staphylococcus spp*. and *Enterococcus spp*. are associated with musculoskeletal diseases in broilers. A potential source of these pathogenic bacteria in broilers is vertical transmission from the breeder hen. The age-related presence of these bacteria may be an indication of possible risk-factors associated with vertical transmission of broiler pathogens.

Cross-protective Bacterins for *E. coli* and *Salmonella*

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Despite the fact that they are among the most studied pathogens known to man, *Salmonella* and *E. coli* have resisted many disease control measures. *E. coli* is one of the most costly bacterial pathogens in poultry and *Salmonella* is an economic challenge to control because of its food safety importance. These gram-negative bacteria have evolved a variety of surface molecules that exhibit a large antigenic diversity which enables them to persist as commensals in vertebrate hosts. The terminal O-antigen of the lipopolysaccharide is one of the most antigenic molecules possessed by the bacteria, but it is also one of the most variable. Similarly, flagella make up a large proportion of the surface molecules present on these bacteria, but it too is very variable. The variability of these molecules has been exploited to create a serotyping system for isolate grouping and epidemiological studies which illustrates the vast array of antigenic types present among *E. coli* and *Salmonella*. However they do possess antigenically conserved molecules within the core LPS which can be exploited to design bacterins with cross-protection. Similarly there is serological conservation among some flagellar antigen types within serotypes of *Salmonella* which offers an opportunity to utilize bacterins to control dissimilar O-serogroups. Current literature regarding cross-protection in *Salmonella and E. coli* will be discussed.

Quantifying antimicrobial use in poultry production

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With an ever-increasing focus on the development and spread of antimicrobial resistance, there is a need to better understand the ways in which antimicrobials are used in human and veterinary medicine. Few data exist regarding specific antimicrobial use practices in animal agriculture. As has been described previously, simple comparison of gross use estimates of different antimicrobials is of limited value due to differences in potencies, duration of activity, relative effect on target and commensal bacteria, and mechanisms of resistance. The objective of this project was to estimate the quantities of different antimicrobials used in poultry production (broiler, turkey and layer) for specific indications and applications. A survey of production companies using a top-down approach to determine the range of antimicrobial usage practices across the industry was conducted. We performed calculations with input data including number of birds and disease incidence in a flock, feed and water intake per day, dose of the antimicrobial in the feed and water, and duration of administration. The calculations provide usage estimates that reflect available data related to specific production practices and industry norms.

Antimicrobial Resistance: Clues Policy Changes Won't Affect the Outcome.

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The contribution from antimicrobial use in food-producing animals to the overall antimicrobial resistance problem in human medicine has been greatly exaggerated. Implementation of changes to the use of medically important antimicrobials in food-producing animals in accordance with the Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) Guidance For Industry # 209 and 213 and the revised Veterinary Feed Directive (VFD) is schedule to take place in December 2016. As part of FDA-CVM's recommendations, growth promotion uses of medically important antimicrobials will end and uses of all medically important antimicrobials administered by feed or water will require a VFD or prescription by a licensed veterinarian. Medically important antimicrobials will only be used to prevent or treat diseases under veterinary supervision. Although these policy changes are good they are not likely to result in a measurable improvement in antimicrobial resistance in humans since antimicrobial use in food-producing animals is not a significant contributor to the resistance problem in humans. This presentation will use current and past data from antimicrobial resistance databases and other studies on antimicrobial resistance to document that the likelihood of a measurable positive impact on human health from the new policy is from very small to nil.

Examination of the Environmental Reservoir of Resistance Genes for Foodborne Pathogens in Poultry Production

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The role of environmental bacteria in maintaining antimicrobial resistance genes in poultry production environments was examined. Samples were collected from farms and processing plants in Alberta during 2012. Approximately 150 aerobic bacteria were isolated and identified to species. Half of the isolates were gram-positive, and half gram-negative. More than twenty five species were isolated, some of which demonstrated ability to form biofilms in vitro. Approximately 23% of gram negative and 28% of gram-positive bacteria were multi-drug resistant (three or more drug classes). Resistance to antibiotics of very high importance such as ceftriaxone was observed in 35 percent of gram-negative isolates. The *E. coli* isolates expressed the highest level of multi-drug resistance, with ten of fourteen possessing an *Amp*C Cephalosporinase phenotype. These isolates were found to possess plasmids harboring *Bla*-Cmy2 genes. The plasmids conferred a multi-drug resistance phenotype when conjugated into recipient *E. coli* strains. Some plasmids did not require helper plasmids for transfer in culture. The sizes of the resistance plasmids were determined. The incompatibility types of the resistance plasmids was determined using PCR and sequence analysis of the *Inc* locus. The results suggest that multi-drug resistant environmental bacteria present on poultry farms and processing plants may constitute an important reservoir of antimicrobial resistance genes transferrable to foodborne pathogens.

Are *Salmonella* from Poultry Distinct from Those Present in the Environment?

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There are over 1 million cases of *Salmonella* infection annually in the United States resulting in almost 400 deaths. The vast majority of these cases are linked to the consumption of contaminated meat, eggs, and dairy products. However, outbreaks of *Salmonella* linked to produce has shown that water can and does serve as a potential source of *Salmonella* contamination. What contribution do animal manures play in *Salmonella* contamination of the watersheds used to irrigate and process crops? We performed whole genome sequencing on several non-traditional serovars, some of which were isolated from aquatic environmental sources. Various unique genetic mobile elements, pilus and metabolic genes were identified. A large molecular weight, conjugative plasmid was discovered in *Salmonella* serovar Mikawasima and was found to be widely disseminated in our environmental isolates. We also identified a pilus operon that is homologous to the *Escherichia coli* common pilus widely distributed amongst our environmental isolates. Lysogenic phages were also identified but only among a few of the environmental isolates. None of the genes identified were unique to these aquatic environmental isolates as they were also present in poultry *Salmonella*. However, there was a large discrepancy in the distribution of certain plasmid, virulence and metabolic

markers between the environmental isolates and the poultry isolates. It appears that certain *Salmonella* serovars in food animals persist in water due genetic elements common to water-adapted *Salmonella* strains.

Colonization of Internal Organs by *Salmonella* Enteritidis in Experimentally Infected Laying Hens Housed in Enriched Colony Cages at Different Stocking Densities.

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The frequency of human infections with *Salmonella* Enteritidis (SE) has been linked to contaminated eggs and thus to SE prevalence in commercial egg-laying flocks. Contamination of the edible contents of eggs is a consequence of SE colonization of reproductive tissues in systemically infected hens. The animal welfare implications of poultry housing systems have been widely debated, but the food safety significance of laying hen housing remains unresolved. The present study determined the effects of two different bird stocking densities on the invasion of internal organs by SE in groups of experimentally infected laying hens housed in colony cages enriched with perching and nesting areas. Groups of laying hens were distributed at two different stocking densities into colony cages and (along with a group housed in conventional cages) orally inoculated with doses of 10^7 cfu of SE. At 5-6 d post-inoculation, hens were euthanized and samples of internal organs were removed for bacteriologic culturing. SE was recovered at a significantly ($P < 0.05$) greater frequency from hens in enriched colony cages at the higher stocking density than at the lower density from livers (75.0% vs. 51.4%) and ovaries (51.4% vs. 30.6%), However, spleens from hens in enriched colony cages at the higher stocking density were significantly less often positive fo SE than from hens in conventional cages at that same density (90.3% vs. 68.1%). These results suggest that stocking density can influence the susceptibility of hens to SE, but other housing systems parameters may also contribute to the outcome of infections.

The Impact on Intestinal Colonization with *Salmonella enterica* serovar Typhimurium with Partial Replacement of $NaHCO_3$ in Diet

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The University of Georgia

The goal of this study was to determine whether the common practice of partial replacement of NaCl with $NaHCO_3$ in broiler diets increases cecal colonization with *S.* Typhimurium*. Salmonella*-negative day of age broiler chicks were randomly assigned to 2 treatment groups. Treatment 1 had no $NaHCO_3$ and treatment 2 had partial replacement of NaCl with 0.23% $NaHCO_3$. All birds were orally gavaged with 10^4 *S.* Typhimurium (RifR) at 2 days of age. Pen weights and cecal droppings were collected weekly. Blood chemistry, gastrointestinal digesta pH and ceca were collected 14 and 42 days post inoculation. Birds from treatment 2 had higher body weights at 14, 21, 36 and 42

days post-infection (P< 0.05). The treatment groups did not differ with respect to *Salmonella* CFU/g cecal contents on any day post-inoculation (P> 0.05). Blood potassium and chloride concentrations were significantly lower in treatment 2 group on both days 14 and 42 post-infection (P< 0.05). Gastrointestinal digesta pH differed by region of intestine and treatment (P < 0.001) at 14 and 42 days post-inoculation. Significance between treatment groups of digesta pH was only observed in the gizzard of treatment 2 birds at day 14 post-inoculation. This study revealed that the practice of partial replacement of NaCl with $NaHCO_3$ in broiler diets had an effect on bird weight, gizzard digesta pH and blood potassium and chloride values; however, it did not impact cecal colonization with *S.* Typhimurium.

Using Bioluminescent *Salmonella* to Identify Infection Sites that Might Contribute to Contamination of Ground Chicken Meat

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Although numerous measures have been implemented to minimize surface contamination in poultry carcasses during processing, *Salmonella* are still frequently recovered from ground poultry products. The role of internalized *Salmonella* from systemic infections and its impact on bacterial cell numbers in ground poultry are, however, still poorly defined. The goal of this project was to reveal harborage sites for *Salmonella* that might possibly contribute to contamination of ground chicken meat by performing bioluminescence imaging, bacteriology and immunohistochemistry in samples from chickens experimentally infected with bioluminescent (light-producing) *Salmonella* strains. One-day-old specific pathogen free (SPF) chicks were inoculated with 10^8 CFU/0.1 ml of bioluminescent *S*. Typhimurium (bST) or *S*. Heidelberg (bSH) via oral route and euthanized on different time points until day 42 post inoculation. Ground poultry components (neck skin, skeletal muscle with lymphatics, bone and blood) as well as liver, spleen and ceca were aseptically removed for testing. Both *Salmonella* strains were visualized on neck skin samples until day 42 post inoculation and were predominantly observed within the stratum corneum of epidermis, likely as a result of fecal contamination. Some muscle samples had small numbers of bST and bSH as detected by bacteriology and immunohistochemistry in different time points post inoculation and until day 42. Immunohistochemistry revealed *Salmonella* within connective tissue and lymphatics of skeletal muscle samples. Prevalence of bST and bSH in blood and bone was lower and *Salmonella* in these tissues was predominantly detected in the first 2 weeks post inoculation. The results of this study suggest that surface contamination of skin with *Salmonella* attached to epidermal keratin might be a more significant source of *Salmonella* in ground poultry than internalized bacteria from systemic infections.

The Importance of Data in *Salmonella* Risk Mitigation: Development of a Cloud-based Technical Platform for Food Safety Management in Poultry Production

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Foodborne disease outbreaks represent an ever-present risk to human health and the poultry industry, with notable *Salmonella* outbreaks occurring in recent years. Outbreaks result in adverse health effects to the consumer, as well as negative brand impact and significant financial losses to companies. Following a multistate outbreak of *Salmonella* Heidelberg, a California-based poultry producer worked with a biotechnology company to collect, integrate, and analyze data across its operations, from pullet through packaging. Data was initially identified from 2014-2015 for 12,971 flocks, with the ability to trace production from breeder to broiler stage for only 17% of flocks. Less than 2% of broiler flocks with *Salmonella* tests were traceable to *Salmonella* rehang tests in those same flocks at processing. Further investigation increased available data to 22,471 flocks, with 100% flock traceability from pullet through processing for company-owned flocks. Linked flock and *Salmonella* test traceability from broiler to rehang stages improved to 10%. A cloud-based platform was developed to integrate and visualize the company's national-level flock production and pathogen testing data. This provided the ability to examine food safety performance over time at the individual flock level. Operational insights were obtained from the data, which included: ability to trace increased positive *Salmonella* tests at processing back through the production chain; identification of sampling bias at processing; and validation of a new sampling technique with better performance than the carcass postchill test. Visibility into the company's data resulted in real world operational insights and understanding of food safety risk in their production chain.

Utilization of Next Generation Sequencing to Evaluate Vaccination against *Salmonella* Typhimurium in an US Broiler Integrator

John El-Attrache, Roxana Sanchez, Melissa Madsen, Ha-Jung Roh and Corey White

Ceva Animal Health

Salmonella Typhimurium is one of the top six most isolated serotypes associated with salmonellosis in humans. From 1998 to 2008, 34% of Typhimurium infections were linked to the consumption of poultry in the US. In the past three years, a decrease in the incidence of these infections may have been associated to a decrease in contamination of poultry and poultry products. The application of *Salmonella* vaccines in the poultry industry may account for this reduction. A continuous monitoring of the *Salmonella* contamination status at different levels of a poultry integrator should follow the implementation of any *Salmonella* control program. Therefore, *Salmonella* testing before and after vaccination is highly recommended to evaluate the effectiveness of a vaccination strategy. We suggest the utilization of next generation sequencing to study the genomic characteristics of *Salmonella* strains circulating in a poultry environment to understand variations that may explain failure or success of vaccine application. Here we present a case of *Salmonella* Typhimurium contamination in broilers carcasses that ultimately originate from breeder flocks receiving a Typhimurium autogenous vaccine in the field along with a standard program against *Salmonella* Enteritidis. *Salmonella*

Typhimurium was not recovered from the farms or the hatchery, however, this serotype was isolated from 25% of *Salmonella* positive samples at the processing plant. The processing plant was in optimal performance when evaluated under the FSIS, USDA standards. The *Salmonella* Typhimuriun strains were compared phenotypically, by standard microbiological methods, and genotypically, by next generation sequencing, to the strain contained in the vaccine. Relevant associations are presented and discussed. These findings aided in the adjustment of the vaccination program and provided information on *Salmonella* characteristics that may play an important role in environmental survival.

Application of Six Sigma Principles and Methodology to Improve Animal Welfare: Reduction in Wing Damage

Kenneth Opengart, Andrew Todd, Charles McIver, Donnie Duvall

Keystone Foods

Six Sigma is a set of techniques and tools used for process improvement which seeks to improve the quality of the output of a process by identifying and removing the causes of defects and minimizing variability in manufacturing and business processes. The six sigma DMAIC (define, measure, analyze, improve, control) project methodology was utilized to study the occurrence of wing damage from the point that broilers are caught on the farm through the point the broilers are shackled at the processing plant. A diverse team of employees from live operations, plant operations and quality assurance worked together with a clear commitment to making decisions on the basis of verifiable data and statistical methods (six sigma principle) to identify primary, controllable causative factors of wing damage with a clear focus on (a) achieving measurable and quantifiable reduction in this key animal welfare KPI and (b) determining the positive economic impact associated with improving sellable pounds. As a result of the six sigma project, key areas attributed to causing wing damage were identified, and processes were put in place to improve, and then maintain, performance.

Development and Implementation of a Progressive Welfare Index (PWI) as a Real-time Indicator of Overall Welfare Performance

Kenneth Opengart, Jeremy Williams, Charles Johnson, Eric Latham, Ronnie Elmore, Chris Beebe

Keystone Foods

Within a standard commercial poultry production environment, animal welfare key performance indicators (KPIs) are routinely assessed and recorded. The frequency of these observations can be weekly if farm-based and as often as hourly if plant-based. Current welfare audits assess overall welfare performance/compliance are performed weekly or monthly. These point-in-time audits provide an assessment which (a) evaluates welfare-based parameters on an infrequent basis, (b) incorporates a finite number of observations of limited key criteria and (c) is performed in an environment where there is full knowledge of audit occurrence which may influence employee/contractor behavior during the audit. The Progressive Welfare Index (PWI) incorporates in-plant welfare assessments (animal holding environment, DOAs, shackler performance, wing damage, leg damage, stunner efficiency, video monitoring, etc.) on an on-going, real-time basis using the Infiniti-QS platform for data collection and integration within a unified data repository, real-time analysis and reporting. The observations that are collected within the Infiniti-QS system are assigned a weighted

value based on the relative importance of the specific KPI to overall welfare condition. The individual values are subsequently rolled into a weighted performance index which defines current system animal welfare performance and predicts final overall welfare condition of the birds at processing and, ultimately, for a finished product to the customer. Hatchery (chick holding, chick injury, etc.), on-farm (environmental quality, culling etc.), and live haul (handling, wing/leg damage, etc.) assessments will be incorporated to provide a comprehensive PWI that reflects the most current, real-time welfare state of the entire welfare continuum within a vertically integrated complex.

Database mining of normal water biochemical paramaters of water in poultry barns in the province of Québec and the effect of different water additives and antibiotics on these parameters.

Jean Pierre Vaillancourt[1] and Danel Venne[2]

1 Faculté de médecine vétérinaire, University of Montreal, 2 Couvoir Scott ltée

A data base of 847 water analysis was statistically analysed and the effect of certain water additives was investigated. Changes in electrolytes and pH can have an effect on the physiology of birds and the efficacy of certain treatments. Knowledge of the normal values and standard deviation can help in selecting the most efficacious treatments and adjust feed nutrients to optimise broiler performance.

Investigating respiratory physiology and thermoregulation in poultry using non-invasive point-of-care methods

Marie Souvestre[1], Mattias Delpont[1], Patrick Verwaerde[1], Daniel Venne[2], Jean-Pierre Vaillancourt[3] and Jean-Luc Guérin[1]

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[3]Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada

Pulse oximetry and capnometry are two methods designed for monitoring respectively O_2 saturation in blood and CO_2 in either inspired or expired air. These methods are routinely used in human and veterinary medicine, including pet bird medicine, mostly for anesthesia monitoring.

In the poultry industry, pulse oximetry has been successfully used in broiler selection, in order to control the incidence of ascites associated with lung hypertension. We assessed the relevance of pulse oximetry, capnometry, associated with infrared thermography for monitoring oxygenation, ventilation and thermoregulation in mule ducks during the force-feeding period, as a model. During the study, ducks did not show any modification of S_pO_2 during the force-feeding period, suggesting a control of respiratory homeostasis. The increase of E_tCO_2, respiratory rate and temperature during this period suggests an increase of the metabolism, as well as an increase in heat-loss, due to an increased thermolysis. Furthermore, these non-invasive methods showed a fair correlation with blood gas analysis tested using a portable biochemical analyzer.

Interestingly, the increase in temperature was mostly observed on the bill and feet palms,

suggesting that bill and feet surfaces are major zones of heat loss during hyperthermia in ducks.

Beyond this duck model, pulse oximetry and capnometry, widely available in veterinary practices and easy to implement on farm, may be relevant for the assessment of respiratory health and welfare of poultry.

Monitoring cleaning and disinfection protocols with the use of AccuPoint as hygiene screening tool in hatcheries

Munoz, R., Ahmad, M and Rice, J.

Neogen Corporation, Lexington KY

Introduction:
Adenosine triphosphate (ATP) is an energy source for all living organisms. The presence of live and/or dead organisms on surfaces, as well as on other material associated with biofilms, can be indicated by the detection of ATP from surface samples.

The AccuPoint Advanced system is a device that detects ATP on surfaces. Using the AccuPoint system, a surface's level of cleanliness is measured in relative light units (RLU) in only seconds. The test utilizes an enzyme system to detect ATP and generate light, and the intensity of the light created correlates to the amount of ATP detected on the surface. The presence of ATP on a given surface is indicative of the cleanliness of that surface.

The eggs and chick contact surfaces in incubators and hatchers are carefully monitored for the presence of microorganisms. Traditional time-consuming aerobic plate count (APC) standards have been established and used routinely for hatchery hygiene monitoring. With its nearly instant results, the AccuPoint Advanced system could become a complementary tool to enhance a more precise and accurate monitoring protocol.

Results summary:
Values over 400 RLU were definitely associated with unclean surfaces — where adjustments to hygiene protocols should be made in order to achieve RLU values lower than 300. The results using the AccuPoint Advanced system were consistent, and took less than 60 seconds.

Pathology of Wooden Breast Disease in Modern Broiler Chickens: A Histologic and Ultrastructural study

Michael P. Babak, Erin M. Brannick, Carl J. Schmidt, Behnam Abasht

Department of Animal and Food Sciences, College of Agriculture and Natural Resources, University of Delaware

Wooden Breast Disease (WBD) is a novel muscle disorder in the poultry industry observed to frequently affect the breast muscles of high-yielding modern broilers
Characterized by extreme stiffening of the breast muscles upon palpation of the pectoral region, WBD is known to result in significant economic loss in the poultry industry, and may potentially cause behavioral alterations and reduced welfare in birds. To examine tissue changes associated with onset and pathogenesis of this disorder, a time-series experiment was conducted using chickens from a high-breast-muscle-yield, purebred commercial broiler line. Birds were raised for a period of six weeks, and breast muscles sampled on a weekly basis from selected birds and processed for light and transmission electron microscopy. Histologic presentation indicated presence of focal single-myofiber degeneration and hyalinization in the second week, preceding inflammatory reaction that started in the third week. Lesions in the fourth

week were generally characterized by multifocal to diffuse muscle fiber degeneration and necrosis accompanied by increased inflammatory cell infiltration. Lesions in the fifth and sixth week were characterized by diffuse muscle fiber damage, fibrosis, fatty infiltration including granulomatous tissue encompassing lipid droplets, and irregular myofiber regeneration. Ultrastructural examination showed fibrosis with dense regular collagen fibers, irregular Z-discs, myofibril splitting, displacement and degeneration, including mitochondrial degeneration. This study therefore demonstrates that WBD exhibits an early onset in modern broilers and appears to assume a progressive course with acute inflammatory phase occurring in the earlier stages and chronic inflammation and fibrosis in the later stages of the disease course.

HTS: A Unique, Global Surveillance System for Monitoring and Benchmarking Enteric Health

[1]Alexandre Zocche, [1]Sara Steinlage, [2]Tara Roberts, [2]Jeff Wilson, [2]Hind Kasab-Bachi, [2]Andreia Arruda

[1]Elanco Animal Health, [2]Novometrix Research Inc.

A large volume of data is produced from surveillance systems in the food animal industry. Benchmarking and data analytics represent tools that can be used to leverage the value of data and inform evidence-based solutions to improve health and welfare, performance, economic return, sustainability, and food safety.

Developed in 1995, Elanco's Health Tracking System (**HTS**) is a unique global surveillance system that captures incidence and severity data on 60 health conditions in poultry. This system involves collection of a representative convenience sample of five birds per flock followed by performing a necropsy assessment. From January to October 2015, 101,385 necropsies from 48 countries were performed. The top five countries represented were: Brazil (20.7% of all necropsies), France (14.0%), United States (10.9%), Peru (9.7%), and Mexico (7.1%).

Among the conditions assessed are 23 measures of enteric health, including necrotic enteritis, coccidiosis (lesions caused by multiple *Eimeria* spp.), hyperemia, and others. These conditions are used to evaluate the cumulative impact of enteric disease by calculating an Intestinal Integrity (**I2**) index. This index is an important tool which allows producers to: monitor enteric health; perform benchmarking over time, between farms, and against industry averages; and inform and evaluate changes that would improve enteric health and hence profitability.

Integration of HTS with additional industry data, e.g. performance and management, using a 'Big Data approach' will increase the capacity of this system to address industry issues. Preliminary analysis of integrated HTS and performance data has revealed significant relationships between the I2 index and performance outcomes.

A Highly Stable, Water Soluble Fenbendazole (Safe-Guard® AquaSol) for the Treatment of Gastro-intestinal Nematodes

Blayne Mozisek

Merck Animal Health

Intestinal parasitic worms are a common problem in the poultry industry. These parasites have a significant impact, contributing to the transmission of disease, decreasing yield and, subsequently, increasing costs. Parasitism adds to the total

price of production and results in significantly lower producer profits. Due to the systematic impact of intestinal worms, a water soluble fenbendazole (Safe-Guard® AquaSol) has been developed. This new product is a highly stable, farm-friendly, water-administered suspension. The stability of Aquasol does not require frequent agitation and provides a high level of efficacy as demonstrated in repeated animal tests. Gastro-intestinal nematodes including Ascaridia galli (L5 and adult stages) and Heterakis gallinarum (L5 and adult stages) are susceptible. A review of stability characteristics over time and efficacy in layers, broilers, and breeders will be discussed.

Comparison of Specific Intestinal Wet Mount and Histological Findings

Stephen R Collett and Susan M Williams

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

Routine clinical assessment of gastrointestinal tract health has for a long time been primarily focused on coccidiosis control. Within the last decade there has been increasing consumer pressure on production companies to remove in-feed antimicrobials traditionally used to control the direct and indirect effect of coccidiosis on enteric health. In addition a rapid improvement in genetic potential for growth rate and feed conversion efficiency and a rise in the cost of poultry feed. In order to facilitate timely intervention there is an obvious need to expand on and improve the speed, sensitivity and predictive value of routine intestinal health surveillance.

Clinical intestinal health scoring, microscopic wet preparation examination and histological examination was carried out on the gastrointestinal tract of birds during a 6 week coccidiostat/antibiotic alternative trial. One bird per pen, from six pens per treatment, were examined weekly, from one through six weeks of age. The accuracy of specific wet preparation examination findings was evaluated relative to histological findings.

Development of real-time PCR reagents to identify Salmonella DNA in enriched cultures

Kristin Mesires, Lisa Gow, Lori Plourde, Felipe Navarro, Valerie Leathers, and Michael Angelichio,

IDEXX Laboratories Inc., One IDEXX Drive, Westbrook, ME

Rapid identification of Salmonella in the poultry environment is vital for the protection of flock health. IDEXX has developed real-time PCR reagents for the identification of Salmonella DNA in enriched cultures. These reagents include primers and probes to identify *Salmonella spp*, *Salmonella enterica* serovar Enteritidis (SE), and *Salmonella enterica* serovar Typhimurium (ST). In addition, IDEXX has developed a multiplex *Salmonella* spp. and *Salmonella* Enteritidis mix. All primer / probe mixes include an internal amplification control to monitor for inhibitors that may be present in the reaction. These mixes are part of the RealPCR™ modular reagents and can be run using the standard RealPCR™ master mix and on the same cycling program as all other RealPCR™ RNA and DNA targets. The RealPCR Salmonella reagents have been evaluated using characterized samples and synthetic oligonucleotides. The primer / probe mixes have an analytical sensitivity of ≤ 10 copies / reaction for *Salmonella Spp*., SE, and ST with efficiencies of > 95% over at least a 6-log range. Moreover, the *Salmonella Spp*. /SE multiplex target mix successfully detects 10 copies / reaction in the presence of 10^7 copies

of Salmonella Spp. Preliminary data on enriched cultures will be presented.

Immunohistochemical Characterization of Jejunal Epithelial Cell Populations in Young Turkeys with Depressed Growth

Rebecca E. Jones[1], Michael P. Martin[1], H. John Barnes[1], Liara M. Gonzalez[2]

1. Poultry Health Management Program, Department of Population Health and Pathobiology, North Carolina State University
2. Department of Clinical Sciences, North Carolina State University

Poult enteritis complex (PEC) is a poorly defined infectious disease of young turkeys that causes depressed growth. Affected birds are restless, develop watery diarrhea, and, paradoxically, have increased jejunal length and weight. Economic losses result from affected flocks requiring more feed and a longer time to reach processing weight. Annually, our teaching flock (PEC -) at the College of Veterinary Medicine (TAU) is compared with their hatchmates (PEC +) on a commercial farm (COM). In a previous study, compensatory hyperplasia of crypt epithelium was identified as the reason for increased jejunal weight and length. Even with the increased jejunal tissue, jejunal efficiency (body weight/jejunal length) was not maintained and depressed growth occurred. A similar disorder, short bowel syndrome, occurs in people and other mammals following intestinal resections.

The present study characterized cellular dynamics involved in compensatory hyperplasia. Body weight and jejunal weight and length were measured between days 0 and 35. Jejunal samples collected in formalin were transferred to 70% ethanol at 24 hours and refrigerated to use for both H&E and immunohistochemical staining. Specific antibodies identify the number of stem and progenitor cells within jejunal crypts (identified by SOX9) and ratio of jejunal apoptotic cells to cells in the G2-M stage of replication (identified by CASP3 and PH3 respectively). Data are still being analyzed; however, birds in the COM flock averaged 1067g less than TAU turkeys when they were processed. Immunohistochemical findings will permit the cellular fluxes in PEC to be better defined.

Comparison of Specific Intestinal Wet Mount and Histological Findings

Stephen R Collett and Susan M Williams

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

Routine clinical assessment of gastrointestinal tract health has for a long time been primarily focused on coccidiosis control. Within the last decade there has been increasing consumer pressure on production companies to remove in-feed antimicrobials traditionally used to control the direct and indirect effect of coccidiosis on enteric health. In addition a rapid improvement in genetic potential for growth rate and feed conversion efficiency and a rise in the cost of poultry feed. In order to facilitate timely intervention there is an obvious need to expand on and improve the speed, sensitivity and predictive value of routine intestinal health surveillance.

Clinical intestinal health scoring, microscopic wet preparation examination and histological examination was carried out on the gastrointestinal tract of birds during a 6 week coccidiostat/antibiotic alternative trial. One bird per pen, from six pens per treatment, were examined weekly, from one through six weeks of age. The accuracy of specific wet preparation examination findings was evaluated relative to histological findings.

Gangrenous Dermatitis in Turkeys Associated with Used Litter in Extended Dormancy

Sam Hendrix, DVM, Daniel Moore, PhD, Stephen Davis, DVM

Colorado Quality Research Inc., Wellington, Colorado

The ability of historical litter used in previous *Clostridial* dermatitis studies to cause gangrenous dermatitis in adult turkeys after an extended period of dormancy was evaluated. Used litter from gangrenous dermatitis model development work has been used successfully to create a repeatable model utilized to evaluate various preventive and therapeutic options to control outbreaks of the disease in the turkey industry. This litter has been utilized since 2009 to induce up to 50% mortality in a non-treated, control population of adult turkeys. It was last utilized in August/September of 2014. Approximately 3 months after study completion litter samples were taken to evaluate *Clostridial* species profiles. *Clostridium perfringens* was identified in all pens, with *Clostridium septicum* additionally identified in pens with the highest levels of mortality. The litter was allowed to sit dormant without any litter treatment or manipulation for 13 months, at which time 80 excess 13 week old Nicholas 700 tom turkeys were placed on the litter under normal housing conditions. Gangrenous dermatitis mortality was identified within 48 hours of placement on the litter. Litter samples were again obtained to identify and quantify *Clostridial* species identified within the litter. Results, conclusion, and discussion of these findings will be presented.

Comparison of the proteins produced by severe necrotic enteritis (NE) producing strains of *C. perfringens* (*netB* positive) to those of a *netB* positive non NE producing strain

Joan A. Smyth and Neha Mishra

Department of Pathobiology & Veterinary Science, University of Connecticut

Necrotic enteritis (NE) is a severe disease of chickens and turkeys. It costs the poultry industry nearly $2 billion dollars a year worldwide due to reduced feed conversion, carcass condemnation in the slaughter house, medication and treatment costs, and loss of birds that succumb to the disease. NE, which is caused by *C. perfringens,* had been well controlled for many years by use of the so-called 'antibiotic growth promoters' (AGPs) in feed. However, use of AGPs in poultry production has been banned in many countries and is under threat in the U.S. There is an urgent need to develop alternative strategies to control NE. To develop alternative strategies, understanding of the bacterial factors that contribute to virulence, is important. *C. perfringens* has a large genome and produces many proteins. Approximately 10 proteins have been investigated to date as potential virulence factors with respect to NE. Of these, NetB appears to be the strongest candidate for virulence. However, we have a *netB* positive strain which produces active NetB toxin, but which is non-virulent. So while NetB appears to be essential for virulence, some other as yet unidentified virulence factor appears to be involved. We are examining the entire proteome of three severe NE producing strains (*netB* positive) as well as our unique *netB* positive non NE producing strain of *C. perfringens*. In total, 2,424 proteins were identified. The proteins are being compared both qualitatively and quantitatively, for

differences between disease producing and non-disease producing *netB* positive strain.

Feeding Tributyrin or Organic Acid Blend with Essential Oils Administered in the Drinking Water to Broilers to Reduce Clostridium perfringens Induced Necrotic Enteritis

Greg Mathis[1], Charles Hofacre[2], and Richard Sygall[3]

[1]Southern Poultry Research, Inc., [2] The University of Georgia, Poultry Diagnostic and Research Center [3] Perstorp BU Feed & Food

The objective of the floorpen study was to determine the benefit of feeding tributyrin (an ester of butyric acid and glycerol), Bacitracin Methlyene Disalysalate (BMD), or Prophorce® (an organic acid blend with essential oils) administered in the drinking water to broilers in order to reduce Clostridium perfringens(CP) induced Necrotic Enteritis. The treatment groups were: No additive, no CP (NM), no additive; CP (NMI); tributyrin (starter 500g/mt, grower/ finisher 250 g/mt); or the antibiotic BMD (50g/t) both fed continuously in feed; or Prophorce® (1 kg/1,000 liters of water) for 14 days as the sole water source at first necrotic enteritis mortality. A randomized block design with 8 replications of 50 birds per pen was used. All chicks were vaccinated at hatch with a commercial coccidia vaccine. On d19, 20,21 all birds, except T1(NM: no additives) were challenged with Clostridium perfringens (1 X 10^8 cfu/bird). On d21, five birds per pen were scored for NE lesions (scoring 0-3). This study reproduced clinical necrotic enteritis (7% NE mortality for NM) necrotic enteritis. The adjusted feed conversions at all weigh periods dD0-21, 0-35, and 0-42 were significantly improved ($p<0.05$) for Tributyrin and Prophorce® compared to NMI. Final average weight gains (d42) for the Tributyrin and Prophorce® were not statically different from each other or from NM. Prophorce® final weight gain and NE lesions were significantly better than NMI. Thus both Tributyrin in the feed and Prophorce® in the drinking water reduced Necrotic Enteritis in broiler chickens.

Impact of controlling bacteria in feed on broiler performance during a Clostridial challenge. K.E.

Richardson[1], C. L. Hofacre[2], G. Mathias[3], and B. Lumpkin[3].

[1]Anitox, Lawrenceville GA [2]Poultry Diagnostic and Research Center, University of Georgia, Athens, GA, [3]Southern Poultry Research, Athens, GA,.

The removal growth promoting antibiotics and anti-coccidials from feed has resulted in an increase in the incidence of Clostridia associated problems in broilers. Improving biosecurity programs at the farm has often proved successful in reducing this risk. The purpose of this study was to determine if reducing microbial contaminants in feed would also reduce the impact of Clostridia on performance. Ross x Ross day old broiler chicks (male) were into 32 identical floor pens (50 chicks/pen). Pens were blocked by location and randomly assigned to treatment. There were eight replicate blocks/treatment. Treatment groups were negative control (no Clostridia challenge, no feed treatment), positive control (Clostridia challenge, no feed treatment), negative control (no Clostridia challenge) with feed treatment (chemical preservative) and positive control (Clostridia challenge) with feed treatment. Feed (starter, grower and finisher) was assayed for microbial contaminants (mold, aerobic bacteria, enterobacteriaceae and Clostridia). On day 14, birds were orally inoculated with 5,000 oocysts of E. maxima to predispose the bird to Clostridia. On day 19-21, the positive control and the treated feed treatment groups were

intubated with 10^8 cfu of C. perfringens daily. On day 42, the trial was terminated and the impact of treatment on broiler performance (body weight gain, feed conversion, and mortality) determined. It was observed that reducing the level of microbial contaminants in the non-challenged and challenged birds improved feed conversion efficiency, but did not impact body weight gain or lesion scores.

2015 Bacterial Enteritis Global Impact Assessment

Alexandre Zocche[1] DVM, MSc, David Heckman[2] DVM

Elanco Animal Health

The 2015 Bacterial Enteritis Global Impact Assessment (BEGIA) was a survey of 337 poultry experts representing the major poultry-producing regions of the world. Respondents from a variety of disciplines across the industry were asked 25 questions on incidence, impact, treatment and attitudes about bacterial enteritis (BE).

The 2015 survey found that BE is still very prevalent: 74% of respondents reported diagnosing BE at some time in their flocks, and 78% were currently experiencing at least one form of BE in their operation. 64% of respondents felt the BE issue will remain the same or worsen in the next five years.

BE also affects profitability, with 91% of respondents reporting some performance loss caused by BE. Respondents seem to be initiating treatment sooner in the disease state, possibly as a way to offset these potential economic losses. The gap between when respondents say economic damage begins and when treatment should initiate has narrowed to 5% (compared to 12% in 2010).

More than 75% of respondents reported that their end-customers (slaughterhouses, exporters, retailers, etc.) prefer a preventive approach to managing disease. About half of respondents prefer water treatment and feed additives for BE prevention. Over half of respondents expect to have less access to antibiotics over the next five years.

The 2015 survey results highlight that BE continues to be a major concern for poultry producers, affecting the economic potential of their operations. Survey responses point to a need for more options to prevent BE in order to maintain Intestinal Integrity in flocks and protect profitability.

Study on the prevalence and association of a novel *Mycoplasma sp*. with reproductive disease in commercial goose breeders.

Silvia Carnaccini[1], Naola M. Ferguson-Noel[2], Richard P. Chin[3], Mark Bland[4], Tiffany Santoro[1], Arthur A. Bickford[1] and C. Gabriel Sentíes-Cué[1].

[1]California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis, Turlock branch [2]Department of Population Health, The University of Georgia [3]California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis, Tulare branch [4]Cutler Associates International

From April 2014 to May 2015, poor fertility persisting in the goose breeders of a major commercial operation in California was the reason for the submission of both affected and non-affected geese to the CAHFS, Turlock branch (UC Davis). The Toulouse breed was principally affected and fertility dropped from 65.7% to 33.9% at the beginning of the 2014 breeding season. The Toulouse flock

consisted of 410 adult birds, 90 males and 320 females, between 2 and 5 years of age. Inspection of the flock revealed that 44.4% of the Toulouse ganders had severe phallic alterations which prevented them from mating. Severe yellowish caseous exudate was disrupting the normal architecture of the phallus. Microscopically, multifocal lymphoid nodules were infiltrating the submucosa of the phallus and its annexes suggesting Mycoplasma infection. *Mycoplasma spp.* were isolated from the phallus, cervix and cloaca of affected and non-affected birds. Polymerase Chain Reaction (PCR) protocol targeting the 16S-23S rRNA intergenic spacer (ISR) regions and the RNA polymerase beta subunit (rpoB) gene were performed and three distinct species of Mycoplasma were identified upon sequencing and NCBI BLAST analysis: *Mycoplasma cloacale*, *Mycoplasma anseris*, and a novel *Mycoplasma sp.* This was the first report of these mycoplasma species associated with reproductive disease in ganders in the United States. Therefore, an experimental polymerase chain reaction with primers specific for the novel *Mycoplasma* sp. was developed and used to determine its prevalence in the flock. The other mycoplasmas were also tested by regular PCR. The results will be presented.
(The full article will be submitted to Avian Diseases)

Mycoplasma synoviae Antimicrobial Susceptibility Testing

Naola Ferguson-Noel and Xiaowei Gong

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Mycoplasma synoviae (MS) is a poultry pathogen of worldwide prevalence. The current approaches to MS control include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. Antimicrobial therapy may be applied in situations where positive flocks cannot be eliminated, to slow mycoplasma infection, to reduce transmission of the organisms (vertically and horizontally) and to treat clinical signs. The development of antibiotic resistance, costs, regulations and feasibility limit the ability of producers to apply antibiotics, these factors along with the trend toward limited and judicious use of antibiotics emphasizes the need to define optimal treatment and its true effect on MS infections. The clinical predictive value of *in vitro* susceptibility tests (such as minimal inhibitory concentrations (MICs)) may be limited and the necessity of isolating a pure culture means a significant time delay between collection of samples and a final result. The correlation between *in vivo* effect (quantitative real-time PCR) and MICs from recent MS isolates was previously evaluated; PCR protocols for the rapid identification of ribosomal modifications associated with resistance to antimicrobials used to control avian mycoplasma infections were developed to better, and more quickly, predict the likely clinical outcome of medication.

Evaluation of Infectious Laryngotracheitis CEO Vaccine in *Mycoplasma synoviae* positive Broilers

Victoria Drouet Pratt, Naola Ferguson-Noel, Maricarmen García, C. Stephen Roney, Marianne Dos Santos, Ruth Wooten, Tyler Gamble, and D.G. Sandu

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Mycoplasma synoviae (MS) and infectious laryngotracheitis virus (ILT) are common infections seen in the commercial poultry industry in the southeastern United States.

These infections can cause multimillion-dollar losses for the industry. Our goal in this study was to evaluate the impact of a commercial ILT CEO vaccine in MS infected commercial broilers. Groups of broilers were inoculated with a recent MS field isolate via intra-tracheal and intra-airsac routes. One week later broilers were administered a commercial ILT CEO vaccine via eye drop. Clinical respiratory signs were scored at 3 and 5 days post ILT vaccination. The groups were grossly assessed for severity of airsacculitis at necropsy (at 7 and 14 days post ILT vaccination); tracheal and lung lesions were evaluated by histopathology. The degree of infection and replication of MS and ILT was determined by qPCR assays. The severity and appearance of clinical signs were markedly different in birds infected with both MS and ILT, as opposed to the groups infected with one pathogen.

Cytokine expression patterns in conjunctiva, Harderian gland and trachea after ocular or oral inoculation with a virulent strain of infectious laryngotracheitis virus (ILTV)

Gabriela Beltrán[1], Sylva M. Riblet[1], Wanderley Moreno Quinteiro[2], Leah Read[2], Shayan Sharif [2] and Maricarmen García[1]

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Infectious laryngotracheitis virus primarily infects the upper respiratory tract of chickens. The main sites of ILTV lytic replication are the conjunctiva, nasal cavity and the trachea mucosa. We have previously showed that the route of inoculation greatly alters the replication patterns of virulent ILTV strain 63140. When strain 63140 was administered via the ocular route viral replication was detected in trachea, conjunctiva and nasal cavity. In contrast when administered via the nasal or oral routes replication was limited to the nasal cavity. The nasal cavity, conjunctiva and the Harderian gland are structures that although not anatomically connected to the respiratory system are the first to come in contact with the virus and contain associated lymphoid tissues which play essential roles in induction of local immune responses. The specific objective of this study was to determine how the route of inoculation of virulent ILTV strain 63140 influenced cytokine and Toll like receptors (TLR) gene expression in conjunctiva, Harderian gland and trachea tissues after ocular or oral inoculation. Relative quantification of host gene expression for type 1 interferon (IFN alpha and beta), type II interferon (IFN gamma), interleukines IL1b, IL6, inducible nitric oxide (iNOS), TLR3, and TLR21 was performed by reverse transcriptase real-time PCR. Preliminary analysis shows that six hours post oral inoculation significant down-regulation of IFN-alpha and IFN-beta gene expression was detected in trachea of infected chickens. While in trachea of chickens inoculated via the ocular route, 12 hours post –inoculation significant down-regulation of IFN-alpha, IFN-gamma, IL-1b, and TLR3 gene expression was detected. Further analysis of cytokine gene expression in Harderian gland and conjunctiva will be presented.

Serologic monitoring (vaccine take) after Innovax ILT (rHVT-ILT) vaccination using conventional type of ILT Elisa kits and an ILT gI specific protein Elisa kit.

Rik Koopman DVM

Global Poultry Business Unit MSD AH

Measuring the serologic response 2-3 weeks post vaccination is common practice in many countries to evaluate the ILT vaccine take. With the introduction of the new type vector vaccines, where immunity building is based on one ore few parts of the ILT virus that are engineered in a vector vaccine, the question is whether we measure a similar response compared to conventional CEO type of ILT vaccines. Innovax ILT is a vector vaccine (rHVT-ILT) based on a HVT vector carrying 2 immunogenic parts (gI&gD) of an ILT virus inducing protection against mareks and ILT. Advantage of vaccination with the HVT-ILT vaccine is that there is no full ILT virus involved, result no spread of vaccine virus and also prevention of latency and reversion to virulence of the vaccine virus. The goal of a well performed Innovax ILT vaccination is that all individual birds receive the vaccine during the hatchery injection from where immunity induction can start. We have to realize that using this type HVT-ILT the ILT immunity development is depending on the replication of the HVT vector and vaccine application is crucial “missed means missed”. To monitor the quality of vaccination serologic testing by using the Elisa system is common practice. The question is whether conventional existing ILT Elisa kits will be able to pick up the serologic response induced by the gI and gD protein after using Innovax ILT.

Therefore a comparison was set up to test blood samples coming from Innovax ILT vaccinated birds in 3 different Elisa kits, 2 conventional ILT Elisa kits and a specific gI protein Elisa kit.

Samples will be obtained between moment of vaccination and 12 weeks post vaccination.

Pathogenicity of Two Variant Reovirus Isolates from Clinical Cases of Viral Arthritis in Arkansas and North Carolina

Tyler C. Gamble, Erich G. Linnemann, Dulmelis G. Sandu, Victoria A. Drouet, Charles S. Roney, Susan M. Williams, Holly S. Sellers

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Since late 2011, there has been an increased prevalence of viral arthritis and tenosynovitis in broilers reported across the United States. During this time, a concurrent rise in variant reovirus isolation occurred, and these isolates were grouped into five genotypic clusters. A gap in knowledge exists between genotypic characterization and pathogenicity of variant reoviruses. In an effort to better understand the pathogenicity of variant reoviruses belonging to genetic clusters three and four, a clinical isolate from each of the two clusters was selected for study. At day of hatch, specific pathogen free White Rock broilers were challenged with plaque purified avian reovirus isolates at $10^{3.5}$ EID50 by either oral or footpad inoculation routes with one of the two chosen isolates. The birds were placed in pens on clean shavings and monitored daily for clinical signs. Tendon and footpad measurements, cloacal swabs, and weights were collected weekly. At 28 days of age, all birds were necropsied—macroscopic lesions were recorded; tendons, hearts, and intestines were collected for histopathology; bursal weights were measured; and tendons were

individually swabbed for VI and/or RT-PCR. When compared to the control, the cluster three oral challenge group had 7% weight suppression, 5.6% increase in footpad thickness, and 6.7% increase in tendon size at 28 days post-infection. The cluster four oral challenge group, when compared to the control had 10% weight suppression, 9.2% increase in footpad thickness, and 9% increase in tendon size at 28 days post-infection. Histopathology, VI, and RT-PCR are in progress.

Turkey arthritis reovirus; pathogenesis, immune response and genetic profile

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In 2011, turkey reoviruses were isolated from tendons and synovial fluids of >15-week-old lame turkeys displaying swollen joints and occasionally ruptured leg tendons in Midwest, USA. These reoviruses were tentatively called turkey arthritis reoviruses (TARV) to differentiate them from reoviruses isolated from intestinal contents and feces of turkeys namely turkey enteric reoviruses (TERV). TARV were found to be genetically distinct from chicken arthritis reoviruses (CARV). Five experiments were conducted to test the pathogenicity of TARV in turkeys and in chickens and to compare it with that of TERV and CARV. Additionally, this work investigated the virus pathogenesis and cytokine immune responses. TARV showed unique capability to induce significantly higher tenosynovitis scores in turkeys as compared with TERV and CARV which induced minimal scores. Clinical lameness was first displayed at 8 weeks of age in TARVinoculated turkeys at 1 week of age. Lameness in infected group reached approximately 50% at 16 weeks of age. TARV did not induce any lesions in chickens via intratracheal or oral route though the virus was isolated from tendons and internal organs of experimentally infected chickens. TARV inoculation via footpad route induced tenosynovitis in chickens at 2 and 3 weeks PI with no clinical lameness. In pathogenesis study, TARV displayed the greatest replication in intestines and bursa of Fabricius than in leg tendons of turkeys. Viral infection mediated effective antiviral cytokines immune response that limited virus replication in the intestines. Furthermore, viral infection mediated a significantly elevated T helper-1(Th1) cytokine response in intestines and tendons and minimal Th2 and Th17 cytokine response during the early stage (2 weeks) of infection. This work established an experimental model to study TARV which provides early end points that are indicative of disease pathogenicity. Additionally, this work developed a new grading system for histologic tenosynovitis which can be used in a wide variety of experimental models. For lameness evaluation in turkeys, this work developed a grading system for gait scores. In summary, this work showed the unique pathogenicity of the newly isolated TARV and added significant knowledge to TARV pathogenesis and immune response using the newly established reproducible experimental model and the newly developed grading systems for evaluation of tenosynovitis and clinical lameness.

Chick Quality and First Week Mortality Evaluation

Donna Hill

Donna Hill Consulting, Mountain Home, AR USA

To troubleshoot a problem or develop baselines, chick quality at harvest and first week mortality must be evaluated.

A chick quality monitoring or diagnostic program (hatchery) must be developed with the following in perspective:

a. All incubation system provide overall good chick quality.
b. In an egg mass, variations in embryo temperature occur not only in certain areas, but within individual egg flats and hatcher baskets.
c. For this reason, an effective chick quality monitoring program defines the small population of problem chicks that are going to the field.
d. The evaluation should lead to the correct diagnosis of incubator or hatcher involvement.

A first week field mortality evaluation should determine if mortality is caused by field issues, disease, or incubation quality.

Chick defects and chick size will be discussed with the objective of defining issues as incubation (hatcher or incubator), disease or brooding related.

Effects of an Automated Egg Sanitizing Machine using both Hydrogen Peroxide with UV Light to Reduce Bacterial Contamination and Improve Hatchability in Older Hen Flocks

Myles Hill, Josh Lockhart, and Robin Gilbert

Elanco Animal Health and George's Inc.

Two breeder flock sources 62 weeks in age were identified and a total of 1,944 eggs were selected. Three trays containing 162 eggs each were selected for control and treatment from each flock and placed in similar locations within the setter and hatcher. Treatment groups were put through an automated egg sanitizing machine containing both hydrogen peroxide and UV light. Two eggs were randomly selected from each of the three treatment trays from both flocks pre and post treatment and placed on a Rodac contact agar plate. A substantial drop in bacterial load was noted post treatment. On day of hatch, the following parameters were recorded: total and infertile eggs; sums of early, middle and late dead embryos; sums of dead pip, live pip, cull chicks, mal-positioned, contaminated, and abnormal embryos. Treatment effects were significant for percentages of the following: cull and normal chicks, hatch of fertile, total early dead, live pip, and mal-positioned.

Incubation temperature and its effect on chick development and avian pathology

Danuta Furmanek; Ron Meijerhof

MSD AH, Poultry Performance Plus

It has been well known that temperature of the egg during incubation affects the hatch results. More recently it has become clear that also the health status of the resulting hatchling is influenced by the temperature of the egg. Several studies have shown that an egg shell

temperature of 38,9 oC in the second half of the incubation process decreases the heart size with of the hatchling with approx 25% compared to a temperature of 37,8^{0}C egg shell temperature, and that also the mortality due to ascites during the grow out period of broilers increases as a result of this high temperature during incubation. It also has been reported that the immune response against a coccidiosis infection at an age of 5 weeks in layers is negatively influenced by an increase in egg shell temperature during incubation, as well as intestinal development, bone development, bone strength and other parameters. Although the underlying mechanism is not fully understood yet, it is believed that the negative influence of the increased egg shell temperature is caused by an imbalance in nutrient availability for the developing embryo, forcing the embryo to use proteins for the supply of energy instead of carbohydrates. Control of egg shell temperature during incubation is therefore not only important for obtaining hatch results and grow out performances, but also for the health status of the birds.

Creation, Implementation, and its Role as a HPAI Detection Tool in Backyard Flocks

Len Chappell

The Georgia Avian Influenza (AI) Hotline

In addition to Industry meetings, company biosecurity enhancements, public awareness through media campaigns, depopulation equipment upgrades and new foaming equipment purchases, changes in the GPLN laboratory management to accommodate USDA criteria, and other preparation measures, the State of Georgia has established a hotline number to make it easier for backyard owners to seek help with problem flocks and rule out AI in those backyard operations. The AI hotline's development and integration into USDA help sites and the Georgia Department of Agriculture website will be discussed. The involvement of the Georgia County Extensions Service Agents in making backyard flock pre-assessments via the Georgia AI Hotline system will be detailed. The many changes made to the Georgia AI Hotline system, since it was launched, are described.

Use of a Highly Pathogenic Avian Influenza (HPAI) Hot Line to improve early detection of possible HPAI infection in Small (Backyard) flocks in Georgia.

Douglas A Anderson, Louise Dufour- Zavala, James Davis, Len Chappell, Elena Benke

Georgia Poultry Laboratory Network, Gainesville, Georgia

In an attempt to improve early detection of a possible HPAI infection in small (backyard) flocks in Georgia, an HPAI Hot Line was established. The Hot Line was distributed via the Georgia Department of Agriculture, University Extension Service, University Georgia Diagnostic Laboratories, and the Georgia Poultry Diagnostic Laboratory Network. Flock owners would answer questions about mortality, egg production, feed/water consumption, and behavior. Their answers were scored as potential risk of HPAI and results forwarded via the Hot Line. Phone calls were received by trained personnel during business hours and forwarded to responding Clinicians. Calls after hours were directed to GPLN clinical staff via email. The responding clinician would determine risk and a diagnostic plan for detection of AI. Difficulties with the scoring system centered on judgment/interpretation and point values assigned to the clinical signs.

Evaluation of Toe-Trimming Strategies in Commercial Turkey Hens

Michael P. Martin and Rebecca Jones

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

Toe-trimming or 'conditioning' is a common practice in commercial turkey hen production. Toe trimming is performed to prevent scratches that may occur on birds during growth that could lead to welfare issues, increased mortality, and processing condemnations. Genetics and husbandry advances have changed the needs for some management practices. To continue practicing good stewardship and facilitate positive public perception of poultry welfare, poultry practices should be periodically reassessed from the standpoint of welfare and production. An investigation of toe trimming strategies in commercial turkey hens was performed comparing trimming vs. not trimming in three flocks of birds under two different management systems for one commercial breed of turkey. Bird weights and mortality patterns were evaluated. Also, foot pad scoring, digit dimensions, and processing data were evaluated. Data from study will be presented as well as proposed future research directions on commercial turkey hen toe trimming.

Mortality Surveys, An Important Tool to Evaluate Broiler Breeder Health

Jose J. Bruzual and Rodrigo Espinosa

Aviagen, Inc.

Keeping breeder flock healthy is the main responsibility for a poultry veterinarian; in order to do that, we need to understand what the situation in the field is by evaluating the birds in different ways including, looking at the records, visiting the flock at the farm, performing necropsy on site and doing a mortality survey of a particular area or complex on any given day.

Contrary to broiler necropsies that are mostly focused on enteric issues like coccidiosis or necrotic enteritis, broiler breeder issues can involve not just the digestive, urinary and respiratory system but also the musculoskeletal system and the reproductive organs. In addition, broiler breeders can develop metabolic diseases like calcium tetany, peritonitis and sudden death syndrome that can be a consequence of not understanding the bird's needs. It is the veterinarian's responsibility to understand how genetic improvement, proper management and nutrition interact and how this interaction can have an impact on broiler breeder health especially metabolic problems that affect our flocks.

The focus of this presentation is to present the mortality survey as a tool to highlight main issues observed in the mortality of broiler breeders and to use those findings to present the results to the customers in a practical way (including percentage) and understanding the trends in mortality when it goes above what it could be considered normal.

Posters

Antibiotic Resistance/Susceptibiliy

Trends in Antibiotic Resistance to *Salmonella* Enteritidis Phage Types Isolated from Poultry from 2007 to 2015

Thomas Denagamage, Bhushan Jayarao, Valerie Lintner, Subhashinie Kariyawasam

Department of Veterinary & Biomedical Sciences, The Pennsylvania State University

Antibiotic resistance of enteric bacteria in poultry has become a public health issue due to possible transfer of resistance to humans. Objectives of this study were to examine the resistance of *Salmonella* Enteritidis (SE) isolated from diseased birds (broiler and turkey), chicken eggs, and layer environment and to determine if the trend of antibiotic resistance over the years can be explained in relation to the type of poultry and phage types of SE. A total of 205 SE strains (87 layer environment and shell egg, 116 broiler chicken, and 12 turkey) isolated from 2007 to 2015 were obtained from the Animal Diagnostic Laboratory at the Pennsylvania State University. Isolates were subjected to phage typing and tested for susceptibility to a panel of 18 antibiotics. All SE tested were determined to be multi-resistant with resistance to four antibiotics (clindamycin, erythromycin, novobiocin, and penicillin) but were susceptible to ceftiofur, enrofloxacin, neomycin, and tylosin. Further, 92% and 66% of turkey isolates were resistant to two aminoglycosides, streptomycin and gentamicin, respectively which are considered as critically important antibiotics for human use by the World Health Organization. Also, 97% and 77% of the broiler isolates were resistant to sulphadimethoxine and sulphathiazole, respectively. The trends in antibiotic resistance to specific SE phage types will also be presented. These results demonstrate the risk of antibiotic resistance transfer to humans through SE contaminated poultry products and would help poultry veterinarians to make informed decisions regarding the use of antibiotics to treat and control infections due to SE.

Antibiotic resistance profile of *Escherichia coli* isolated from vertebral osteomyelitis of broilers in Brazil

Roselene Ecco[1], Juliana Fortes V. Braga[1], Sylvie Baucheron-Monnier[2,3], Catherine Schouler[2,3]

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Antibiotic resistance profile of *E. coli* strains isolated from nine cases of vertebral osteomyelitis (VO) in broilers from seven different flocks in Southeast Brazil was performed. The strains were tested for 16 antibiotics belonging to eight different classes: aminoglycosides, beta-lactams, cephalosporins, phenicols, polypeptides, quinolones, sulfonamides, and tetracyclines. Most strains, 55.6% (5/9), were resistant to more than three antibiotics and were classified as multidrug-resistant *E. coli*. These MDR profile was characterized mainly by resistance to amoxicillin (100.0%), cefalotin (80.0%), nalidixic acid/ flumequine (60.0%), and enrofloxacin/tetracycline (40.0%). The highest resistance profile was detected to quinolones (average of 40.7%) and beta-lactams

(average of 33.4%), followed by cephalosporins (29.6%), sulfonamides (22.2%), tetracyclines (22.2%), phenicols (11.1%), and aminoglycosides (7.4%). All strains (100.0%) were susceptible to phenicols (florfenicol vet). Regardless of antibiotic class, the highest resistances were detected for flumequine (66.7%), amoxicillin (55.6%), cefalotin (44.4%), and ceftiofur C3G vet (33.3%) and nalidixic acid (33.3%). Our results demonstrated that evolvement of multidrug-resistant *E. coli* is frequent in vertebral osteomyelitis cases, which is a concerning point for poultry industry and public health. It was also possible to note a high resistance to ceftiofur (33.3%), which is increasing when compared with previous studies on *E. coli* in Brazil. On the other hand, susceptibility to tetracycline (77.8%) was higher in comparison with other studies reported in Brazil and other large poultry producer's countries.

Mobility of Antimicrobial Resistance in Avian Pathogenic *Escherichia coli* (APEC).

Catherine M. Logue, Nicolle Lima Barbieri, Tia Cavender, Yvonne Wannemuehler and Lisa K. Nolan

Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University.

Avian Pathogenic *Escherichia coli* (APEC) is a significant cause of mortality, carcass condemnations and economic loss to the poultry industry annually in the US and worldwide. Antimicrobial resistance among APEC is common, often leading to complications in treating flocks where it occurs and causing increased duration of disease and difficulties in its control. This study describes assays to determine the potential mobility of some antimicrobial resistance traits in APEC isolated from the US and internationally. A collection of APEC recovered from production birds diagnosed with colibacillosis was screened for antimicrobial resistance using the broth microdilution (NARMS) and agar dilution assays. Isolates were also screened for plasmid replicon types using PCR to determine the potential location of some of the resistance traits identified with specific interest in resistances that are often plasmid borne including tetracycline, extended-spectrum beta lactams (ESBLs), ampC beta lactamases, and fluoroquinolones. Conjugation assays were carried out using lab strains of *E. coli* under selective and non-selective pressures to determine which resistance traits were mobile and the generated transconjugants were screened for resistance traits and replicon types of the donors. These data demonstrate the potential role of plasmids in the mobility of antimicrobial resistance among APEC and other commensal organisms of production poultry.

Impact of ceftiofur withdrawal from a Canadian hatchery on cephalosporin resistance in extra-intestinal pathogenic *Escherichia coli*

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Ceftiofur, a cephalosporin antibiotic, has been used in hatcheries to prevent early mortality in chicks. Extra-label use of this antimicrobial in poultry was banned in 2012, by the U.S. Food and Drug Administration, while the Canadian poultry industry has implemented a mandatory policy eliminating the preventive use of

ceftiofur at hatcheries from May 15, 2014. Such withdrawal has been correlated in the past with a decline in the prevalence of cephalosporin-resistant *Salmonella Heidelberg* isolates in chicken meat, but the effect of this withdrawal on extra-intestinal pathogenic *Escherichia coli* is unknown. Our objective was to verify if ceftiofur withdrawal at the hatchery decreased the prevalence of cephalosporin-resistant *E. coli* after 12 months.

Two vertical samplings were done, firstly in 2014, prior to ceftiofur withdrawal, and then in 2015, one year post-withdrawal. Samples consisted of feces from 8 broiler breeder flocks, fluff and meconium from matching chicks, yolk sac swabs from omphalitis cases and feces from 4 week-old broiler chickens originating from tested breeders, all samplings being repeated three times. Frozen samples were thawed and enriched in MacConkey broth containing Ceftriaxone (1mg/L) and passaged on MacConkey agar containing Ceftriaxone (1mg/L) to facilitate recovery of extended-spectrum β-Lactamase producing *E. coli* colonies. All isolates were confirmed as *E. coli* by PCR for the detection of housekeeping gene *uidA*.

Out of 80 samples tested in 2014, from various sampling sites and points, all showed at least one *E. coli* colony resistant to ceftriaxone. Samples from 2015 and more from 2014 are currently being processed.

Avian Influenza

A Preliminary Survey of Hemagglutinating Viruses from Ducks in the Province of Buenos Aires, Argentina.

Celina Buscaglia[1,2, 3]

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A surveillance in wild birds was started in October 2008. Samples obtained from ducks: (a)"*Pato Barcino* (Anas flavirostris)" Speckled Teal and (b) a cross of Mallard ducks and Peking ducks that lives in Carilo will be reported. Fecal samples were collected from M. B. Gonnet and the county of Pinamar in the Province of Buenos Aires, Argentina. Samples were pooled according to date. Pooled samples 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. Neither Avian Influenza viruses nor paramixoviruses were isolated.

Litter Composting Procedures for Inactivation of Avian Influenza Virus in Eggs.

Teresa V. Dormitorio, Joseph J. Giambrone, Kenneth S. Macklin

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This study examined optimal litter composting conditions for the rapid inactivation of avian influenza virus (AIV) in embryonated chicken eggs (ECEs). AIV infected and non-infected ECEs were placed into boxes that were then placed either in the middle of a litter compost pile or outside the compost pile. Hourly temperatures were monitored for 72 hours using data loggers. Boxes at designated positions were removed after 24, 30, 36, 48, 54, 60, and 72 hours. The allantoic fluid from each composted or non-composted egg was inoculated into ECEs for determination of AIV survival by the hemagglutination test. Trial 1 showed that there was 62.5% AIV survival at 48 hours of composting, however, the virus was eliminated after 54 hours. In contrast, no viable virus was re-isolated from the eggs after 24 hours of composting in the 2nd trial when the internal compost temperature reached 57^{0}C. Results showed that optimum composting conditions and procedures are required to eliminate AIV in eggs within 24 hours. Litter should be adequately moistened and thoroughly mixed, so that all areas of the compost pile will attain maximum temperature. It is also necessary to bury the infected eggs in the middle of the compost pile since temperature on the top only reached 34^{0}C. At 30^{0}C room temperature, AIV survived for 3 days. Using these results, a more effective composting strategy to rapidly inactivate AIV infected eggs can be implemented.

Understanding New Highly Pathogenic Avian Influenza (AI) Viruses Affecting the U.S. Poultry Industry and their Persistence

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Biosecurity information, feces, bedding material and footbath management is being collected by visits and surveys to poultry producers. The most representative managements will be mimicked in laboratory settings. Low pathogenic (LP) H6N2 and highly pathogenic avian influenza (HPAI) H5N8 will be mixed with feces prior to spike bedding material and footbaths. After spiking the material samples will be collected at four different time points in the case of feces/bedding material and 3 time points in the case of footbaths. The goal is to determine the persistence of the virus in productive settings. Knowing the persistence characteristics of AI virus will help to adapt biosecurity measures to outbreak situations in order to prevent the introduction of the virus into new flocks and further spread.

A Synthetic Vaccine of Biodegradable Microspheres for Controlled Release of Adjuvantand Femtomole-dosed Peptide Antigens Protects Against Infectious Bursal Disease Virus.

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A patented T cell vaccine platform that is based on immunization with low femtoMole doses of antigen peptides rather than whole protein antigens was developed. The antigens are 20-amino acid peptides of a protein that overlap by 10 amino acids, encompassing the complete sequence of a protective protein. These peptides are each used at extremely low quantities (~54 pg/chicken vaccine dose). The delivery platform for this vaccine is a powder of microspheres of 2 µm diameter composed of peptide antigens combined with a matrix of biodegradable poly (lactide-co-glycolide) polymer (PLGA) and the block copolymer adjuvant Pluronic L121®. The vaccine powder can be stored at ambient temperature and is suspended for injection. This low antigen-dose microsphere vaccine can be completely synthetically produced by industrially scalable spray-drying technology and has been proven effective against infectious bursal disease virus. Because of the low amount used, hundreds or thousands of peptide antigens may be incorporated into a vaccine, opening the potential for broadly protective vaccines effective against many strains of a pathogen, or for polyvalent vaccines. A peptide vaccine against infectious bursal disease virus (IBDV), 202 overlapping 20-mer peptides were incorporated that encompassed the complete IBDV proteome. Challenge experiments were performed using between 100-270 mixed-sex broiler or SPF leghorn chickens. Two of the experiments served to optimize the IBDV challenge model using either SPF leghorn or broiler chickens for challenge dose titration and time of challenge and post-challenge euthanasia. In two high-dose challenge infections the initial vaccine formulations were not protective. However, in a low-dose challenge infection advanced vaccine formulations provided 50 and 80% protection.

Cost-effectiveness of avian influenza vaccination strategies in Bangladesh: added value to day old chick vaccination

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Bangladesh is the 4th most affected country by Highly Pathogenic Avian Influenza (subtype H5N1) in poultry (OIE, 2015). Since first outbreaks in 2007, HPAI H5 it has spread dramatically over the country affecting the poultry industry. Since November 2012, a novel recombinant HVT (Herpes virus of Turkey) AI vaccine (Vectormune AI) is being applied in some industrial hatcheries in Bangladesh. The objectives of this study were to assess the cost-benefit of AI vaccination at the hatcheries and the feasibility of implementing AI vaccination of day-old chicks in the different production sectors in Bangladesh.

An epidemiological predictive model has been designed by the French Institute for Agricultural Research for Development to simulate the distribution profile of AI immunity according to the different types of poultry farming, production sectors, and vaccination scenario against Avian Influenza commonly used in a given country. This model has been applied in Egypt, Vietnam and now in Bangladesh. The analysis of the production dynamics and flow of products from the different segments to the market was performed. Comparative cost-benefit analysis was done using marginal costs and monetary benefits to estimate the benefit-cost ratio for each vaccination scenario and poultry production type. AI vaccination strategy in day-old chicks was shown to be more efficient than the current strategy using inactivated vaccines. Further results of this epidemiological study will be presented.

Network modeling of poultry production and immunity levels: Analysis and perspectives for vaccination strategy and control of highly pathogenic avian influenza in Vietnam.

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[1]Ceva Santé Animale, France; [2]Vietnam National University of Agriculture, Vietnam; [3]CIRAD- AGIRs, Montpellier, France.

The objectives of this study were to assess the potential added value and feasibility of implementing Avian Influenza (AI) vaccination in day-old-chicks (DOC) in Vietnam. The Vietnamese commercial chicken production network model was combined with a flock immunity model to simulate the distribution profile of AI immunity according to different vaccination scenarios (including DOC vaccination or not). The model estimated the vaccine coverage for each node of the network and vaccination scenario, the positive sero-conversion levels and the duration of sero-protection.

The model predicted that targeting DOC AI vaccination in integrated hatcheries would significantly increase immunity levels in the overall poultry population in Vietnam (from <20% to >70%). This strategy was shown to be more efficient than the current strategy using inactivated vaccines, but it will not be sufficient to increase the immunity levels of the native bird's population. However, spatial analysis demonstrated that this strategy would significantly improve the protection coverage and therefore disease control in the provinces at higher risk of HPAI infection.

Surveillance of amino acid substitutions in avian influenza viruses isolated from wild birds from South Korea, 2010-2015

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Wild birds are considered natural hosts of influenza A viruses and provide environment to generate genetic diversity to many types of animal and human influenza A viruses. Also, reassortments and point mutations of the gene of influenza viruses especially occur in the host wild birds. Various amino acid substitutions generated by point mutations in the gene of influenza viruses have been known to contribute to replication, pathogenicity, transmission and antiviral resistances to the viruses. Mutated viruses

bring to further potentially severe influenza pandemic that may result from cross-host evolution of the influenza viruses. Therefore, the main objectives of the surveillance were to identify major amino acid substitutions of 8 different gene segments of avian influenza viruses isolated from wild birds and to confirm that wild birds can facilitate replication, pathogenicity, transmission and antiviral resistance of avian influenza virus. Total 62,136 fresh fecal samples were collected from major wild birds habitants from 2010 to 2015 in Korea and 228 avian influenza viruses were isolated, for an overall prevalence of 0.37%. H3N8 were the most frequently detected subtypes and Surveillance of amino acid substitutions in avian influenza viruses isolated is progressing.

The Pathobiology of Highly Pathogenic H5N2 Avian Influenza Virus in Ruddy Ducks and Lesser Scaup

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Southeast Poultry Research Lab, US National Poultry Research Center, US Dept. of Agriculture-Agricultural Research Service

The susceptibility and pathogenesis of avian influenza virus (AIV) has not been characterized in numerous duck species, especially diving ducks, some of which migrate across the continental US. The pathobiology of highly pathogenic (HP) H5N2 AIV was characterized in two diving duck species, Ruddy ducks (*Oxyura jamaicensis*) and Lesser Scaup (*Aythya affinis).* Adult ducks and hatching eggs for both species were obtained from captive breeding colonies at the Patuxent Wildlife Research Center, Patuxent, MD. Adult Ruddy ducks (serologically negative for AIV antibody) were evaluated with exposure to three doses of A/Northern Pintail/WA/40964/2014 H5N2 HPAIV (10^6, 10^4 and 10^2 50% egg infectious doses) and juveniles were only exposed to the highest dose. There were only sufficient juvenile Lesser Scaup to expose to the two highest doses. Virus shed, clinical disease, mortality, gross and microscopic lesions were evaluated. There was no HPAIV mortality or disease in the adult Ruddy ducks or in the juvenile Lesser Scaup. There was 60% mortality in the juvenile Ruddy duck group, but no gross lesions were observed. Virus shed, serology and microscopic evaluation data are pending.

Biosecurity

Biosecurity Risk Survey for GP farms in Latin America

Jose J. Bruzual[A] and Luis B. Gomez[B]

[A]Aviagen, Inc., [B]Merial-Select.

Broiler breeder Grandparents farms need to have high biosecurity standards in order to provide broiler breeder pullets and cockerels free of particular diseases, minimizing the chances of disease transmission to customers. In a way to standardize customer's biosecurity standard and protocols, a biosecurity risk survey was developed based on the model presented by Bruce Stewart-Brown at the DPI meeting in 2005. The objective is to properly address different areas that can put in jeopardy the integrity of the farm. The assessment will cover 4 aspects of the biosecurity included in a total of 76 questions. The questions were related to the location of the farm (23), the farm itself (23), the house within the farm (22) and general procedures that need to be considered (10). One particularity is that questions are graded on a logarithmic scale (1,000, 100, 10 and 1) allowing a big separation among the different categories. The final scoring will allow the farms to be

separated in 5 categories. Excellent/very low risk (1-78), good/low risk (79-780), Average/some risk (781-1,483), Bad/high risk (1,484-7,800) and Dangerous/very high risk (>7,800). Very recently the survey was modified to be used through an automated system that allows to do the evaluation with a phone or a tablet and to have the information electronically available at the time of the evaluation. The point system is very important to address issues with customers and changes overtime.

Blackhead

The efficacy of three hydroxyquinolones against *Histomonas meleagridis* growth in an *in-vitro* assay.

Lorraine Fuller, Greg Mathis*

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Histomonas meleagridis, a flagellated protozoan parasite, is the causative agent of blackhead disease or histomoniasis in gallinaceous birds. Blackhead disease is becoming more prominent issue due to the removal of drugs used in prevention and treatment. Nitarsone (4-nitrophenylarsonic acid) was the last approved preventative drug available in the United States against blackhead disease, and is to be removed from the market in the beginning of 2016. In an effort to discover new uses and repurpose available drugs, three different hydroxyquinolones were tested against *H. meleagridis* growth *in-vitro*. Clioquinol (5-Chloro-7-iodo-8-quinolinol), nitroxoline (8-Hydroxy-5-nitroquinoline), and decoquinate (Ethyl 6-decyloxy-7-ethoxy-4-hydroxy-3-quinolinecarboxylate) were tested for activity in an i*n-vitro* drug assay using dimetridazole and nitarsone as positive controls. At the endpoint of 72 hours, plates were analysed for *H. meleagridis* growth, and compared with the negative control (no drug) as well as both positive controls. Decoquinate suppressed growth at high levels only (10,000 ug/ml), and showed limited activity at 1000 ug/ml. Clioquinol and nitroxoline completely suppressed growth at 1 ug or higher, and were highly effective at 0.1 -0.01 ug /ml. The activity of both clioquinol and nitroxoline are similar in to those of dimetridazole, and substantially better than nitarsone.

Clinical and pathological characteristics of a Histomoniasis (Blackhead) in backyard poultry in Vietnam

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Histomoniasis is a new disease in Vietnam, which was major cause of economic loss in poultry farming in some provinces in Vietnam, such as Thai Nguyen, Bac Giang, Phuc Yen, etc. Observation clinical 120 infected chickens 3 – 4 months, the necropsy in 52 chickens, after that choose 32 chickens had characteristic lesions to fix in 10% buffered formalin, tissue specimens were processed, paraffin-embedded, hematoxilin – eosin (HE) staining and finally used optical microscopy to observe. The results about three topics: clinical signs, gross lesions and microscopic lesions, respectively. The clinical signs had seen in an infected flock may just be anorexia, depression, listlessness, cyanosis of head, drooping wings, unkempt feathers and sulphur-yellow diarrhea. Chickens may be sick

for sometimes and become emaciated before death. The primary gross lesions in caeca were enlarge, marked inflammatory changes and ulcerations, causing a thickening of the cecal wall. The cecal pouches contained a yellowish gray or green, caseous core or “a dry, cheesy core” in later stages. Gross lesions of liver were strongly variable in appearance, enlarged liver spotted with target-like lesions representing areas of necrosis, or cell death with gray in color.Histopathological examination showed typical cecal lesions with the lining of caeca is destroyed, lost completely the structure of the intestinal epithelium. Furthermore, in the caeca were found trophozoites under the layer of exudation. In the liver, microscopic lesions were massive multifocal necrosis and haemorrhages, pink color. In necrosis areas, moderate infiltration of mononuclear cells and multigellated forms of the trophozoite dispersed within the organ. In other areas, liver cells were degeneration and infiltration of many inflammatory cells as Heterophile and Eosinophile. This is the first report of the pathological characterization of H. meleagridis in backyard poultry in Vietnam.

Increased Concerns for the Lack of Therapeutic Interventions against Histomoniasis (Blackhead Disease) in Turkeys

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Histomoniasis (Blackhead disease) is a serious concern for the turkey industry in the United States (US). At least 50 cases of histomoniasis were reported each year since 2007 in the US (2013 USAHA Annual Meeting Proceedings) and mortality in the infected turkey flock can reach 100%. Therapeutic options for the control and prevention of the disease are limited. There is a need for research into the development of new animal drugs and other possible interventions, including vaccines and management techniques for the control and prevention of histomoniasis in turkeys. The purpose of this presentation is to highlight FDA’s interest in exploring with colleagues in academia, the pharmaceutical industry, the poultry industry and others, possible therapeutic interventions against histomoniasis in turkeys, leading to an approved new animal drug to fill this important therapeutic need.

Regulatory Considerations for Approval of Drugs against Histomoniasis in Turkeys and Gamebirds

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The Food and Drug Administration’s Center for Veterinary Medicine (CVM) recognizes that approved therapeutic options for the control of histomoniasis in turkeys and gamebirds are limited. CVM has actively engaged in discussions with drug sponsors about potential regulatory pathways and product development plans for the approval of drugs against the disease. Gamebirds such as pheasants, partridges, and quail qualify as minor species under the Minor Use and Minor Species Animal Health Act of 2004 (MUMS Act). In the case of turkeys, if the number of

turkeys intended to be treated with a new animal drug is less than 14 million, this might be considered minor use under the MUMS Act. Minor species and minor uses in major species are eligible for incentives such as conditional approval, grants to support safety and effectiveness testing, and exclusive marketing rights. The development plan for a new animal drug intended to be used in food animals, such as turkeys, aids drug sponsors in addressing the five major components (*i.e.,* technical sections) required for the approval of a new animal drug: 1) Target Animal Safety; 2) Effectiveness; 3) Chemistry, Manufacturing, and Controls; 4) Human Food Safety (HFS); and 5) Environmental Impact. CVM understands that addressing the HFS technical section is a major concern for sponsors of anti-protozoal drugs. The HFS technical section evaluates microbial food safety, toxicology, and residue chemistry aspects of a new animal drug. This presentation will discuss regulatory pathways and provide information on addressing regulatory concerns for the approval of potential drugs against histomoniasis in turkeys and gamebirds.

Case Reports

Chondronecrosis with osteomyelitis caused by *Enterococcus cecorum* in broiler, Korea

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Bacterial chondronecrosis with osteomyelitis was most commonly caused by *Staphylococcus aureus*, but *Escherichia. Coli and Enterococcus spp.* are sometimes involved before 2000. But recently *Enterococcus cecorum* is considered as an emerging pathogen of broiler flock in many countries. We describe clinical history, pathological findings, and bacterial isolation of a chondronecrosis with osteomyelitis case caused by *Enterococcus cecorum* in broiler. The effected flock was consisted of 105,000 broilers(Cobb), equally divided into three groups of 35,000 birds. Two groups showed clinigal signs(lameness, decrease in flock uniformity, and increased mortality) in July, 2015. The onset of clinical signs were recognized at 13 days old of ages. The flock mortality was ten percent. Macroscopically opacity of pericardial membrane, fracture of femur, and swelling of stifle joint were observed. Microscopically, severe suppurative synovitis was seen in femur and tibia. Chondronecrosis in growth plate, and suppurative osteomylitis in femur and tibia were observed. Moreover fibrinous epicarditis and multifocal necrosis in spleen were seen. The femoral heads were grinded. And then, the novel method was used to measure how many bacteria are in femoral head of affected chickens. *Enterococcus* cerorum (1.03 - $5.7X10^3$ CFU) was isolated. Antibacterial sensitivity test revealed ampicillin, ampicillin/clavulanic acid and florfenicol were sensitive against the isolate. The study on pathogenesis of *Enterococcus* cerorum by experimental inoculation will be needed.

An Outbreak of Adenoviral Tracheitis in Commercial Goslings in France

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The present case report provides clinical, pathological and virological data on an adenoviral tracheitis outbreak in a commercial flock of 6-day-old goslings in France. Clinical signs consisted mainly of acute dyspnea and suffocation followed quickly by death of the affected birds. Total mortality reached nearly 5% over a period of 5 days and no veterinary treatment seemed to be relevant. The only significant macroscopic pathological findings were white opaque plugs in the trachea. Histological examination revealed a severe fibrino-necrotic tracheitis and highlighted significant basophilic inclusion of infected cells suggestive of adenoviral infection. No significant bacterial isolation or fungal growth could be obtained from respiratory tissues sampled. Tracheal samples tested positive for EDS76 (*Duck Atadenovirus A*) PCR. Partial sequencing of the hexon gene confirmed that this virus clusters with *Duck Atadenovirus A* (DAV-A). Similar cases has been clinically suspected on two gosling flocks hatched from the same breeder flock during the same week, but could not be confirmed experimentally. DAV-A is considered as infecting widely duck and geese flocks without any pathological impact. Further investigations are required to clarify the actual incidence of this virus and its pathological significance.

Fowl Tick (Argas persicus) Infestation in a Backyard Chicken Flock

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Cornell University Animal Health and Diagnostic Center, College of Veterinary Medicine

Paralysis in adult chickens can be caused by different etiologies namely, Marek's disease virus, Newcastle disease, Listeriosis (encephalitis), botulism and in rare cases, tick infestations with *Argas persicus*, the fowl tick. Acute deaths in four chickens of mixed breed introduced to an existing flock of eight was reported. According to the owner, the chickens had been in good health without any physical abnormalities but he had owned other chickens showing signs of an ascending paralysis over the course of the year. These chickens had trouble walking but were able to flap their wings initially and would later die after losing control of their wings.

A visit to the farm revealed two coops separated by a wall in a large barn with metal nests attached to the middle wall of the chicken coop. The dark chicken coop was next to a second brighter one housing ducks, turkeys and peafowl. Discoloration of the wood in both coops prompted a closer look that showed large numbers of ticks tightly packed in cracks and crevices. Examination of the chickens revealed heavy tick infestation in the axillary areas and keel. The birds also had a low body condition scores. The ticks were identified as *Argas persicus*, the fowl tick and the owner was advised to treat the surviving chickens and the coop with suitable insecticides.

The Fowl Pox Cases with Co-Infection of Infectious Laryngotrachitis Virus in Chicken

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Fowl Pox (FP) and Infectious Laryngotracheitis (ILT) are economically important diseases of chicken that have been recognized in many countries. Also, there were several cases in the chickens simultaneously infected with FP virus and ILT virus. Three cases of layer with severe respiratory signs had been submitted for diagnosis at Avian Disease Laboratory, Chungbuk National University, South Korea. During the necropsy, various samples were collected and tested for diagnosis using virus isolation, polymerase chain reaction (PCR) and histopathology. From these cases, ILT and FP viruses were isolated and confirmed by PCR and sequence analysis. Also, intranuclear and cytoplasmic inclusions were found in the same case by histopathologic examination. Now we try to identify the insertion of reticular endotheliosis virus in the FP viruses isolated from these cases.

HPAI H5N8 Outbreak in Commercial Pekin Ducks in California

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CAHFS- Tulare and Davis Branches, University of California, Davis and CDFA, Tulare, California.

In February of 2015 HPAI H5N8 was diagnosed in 4-week-old commercial Pekin ducks in a flock 10,000 with a history of depression, lethargy, neurological signs and increased mortality. Necropsy of six live ducks revealed a few pale foci in the liver and on the epicardium and thickened cloudy abdominal air sacs in two birds. Histopathology revealed encephalitis, myocarditis, pancreatitis and hepatitis and immunohistochemistry confirmed AI nucleoprotein in these organs. Most of the sera taken from ten live birds were positive for AI by AGID. qRT-PCR confirmed AI in the cloacal swabs, livers, spleens, lungs and pancreas from a few birds

Tetratrichomoniasis-associated Mortality in Four Ducks and One Pheasant at an Avian Conservation Park in Louisiana.

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In 2015, five captive birds died from systemic tetratrichomoniasis in a private avian conservation facility in Louisiana. Between June and October, two African Pygmy Geese (*Nettapus auritus*), one Scaly-sided Merganser (*Somateria fischeri*), one Spectacled Eider (*Somateria fischeri*), and one Koklass Pheasant (*Pucrasia macrolopha*) were submitted to Louisiana Animal Disease Diagnostic Laboratory for postmortem examination. These birds died with necrotizing to granulomatous typhlitis, hepatitis, pneumonia, splenitis, and/or encephalitis. Numerous intralesional protozoal trophozoites were detected in the histologic sections of cecum, liver, lung, spleen, and/or brain of the affected birds. Genetic sequences (approximately 370 base pairs) of the frozen liver samples from two of the birds were identical to the published sequences of *Tetratrichomonas gallinarum*.

T. gallinarum is a trichomonad flagellate protozoan parasite commonly isolated from the cecum of galliform and anseriform birds. The pathogenicity of *T. gallinarum* is controversial. Experimental infections have often failed to produce disease in poultry; however, sporadic fatal cases have been reported in captive and wild birds. One of the five birds died despite a prophylactic treatment with an antiprotozoal agent, ronidazole. To our knowledge, this is the first report of fatal tetratrichomoniasis in both anseriform and galliform birds in the same facility and a failure of an antiprotozoal treatment.

Diagnostics

Frecuency of Swollen Head Syndrome in broilers from Peru

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Avian Metapneumovirus was isolated in 28 broilers flocks in the central coast of Peru. All cases were diagnosticated at the Laboratory of Avian Pathology during 2015 and 2016 year period. All positive chickens to swollen head syndrome (SHS) showed the following clinical signs: facial edema, sneezing, conjunctivitis and depression. Samples of nasal turbinates, trachea, and lungs were collected to perform the molecular diagnostic and serology tests. A total of 28 out of 159 respiratory cases were confirmed to SHS by RT-PCR. Compare to Infectious Bronchitis with 20 out of 159 respiratory cases, SHS is one of the most frequent respiratory diseases in the field. Risk factors as elevated temperature and deficiency ventilation have boosted the numbers of cases in recent years. The serology and molecular tests data is still collected up to december this year.

Specificity Study of Elisa Kits

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Because GPLN is often asked how kits from one vendor compare to those of another, the laboratory decided to perform specificity studies on certain kits from a number of suppliers of ELISA kits. The data from that comparison is the basis for this presentation. This information enables the Laboratory to choose kits that best fit the needs of its customers. Often customers are concerned when screening tests provide positive results and confirmatory tests, which are accepted as the "Gold" standard, yield negative results. Sometimes these findings are due to an early infection which the confirmatory test does not detect, but many times it is due to a lack of specificity of the screening ELISA kits. This lack of specificity can result when antigens coated on ELISA test plates are not purified or treated to remove proteins that can nonspecifically bind to serum components. In this study we attempt to define those kits that do not provide the most accurate information regarding the presence of "specific" antibodies in chicken serum. To perform this study, monospecific antisera were ordered from a commercial supplier. The monospecific antisera were: Avian Encephalomyelitis, Avian Reovirus, Chicken Anemia Virus, Infectious Bronchitis Virus - Mass, Infectious Bursal Disease, Infectious Bursal Disease Variant E, Mycoplasma gallisepticum, Mycoplasma synoviae, and Newcastle Disease virus. Negative Serum was also included in the study. The sera were added to multiple wells of three different ELISA plates of each of the agents studied from each

supplier. The test results were analyzed for reproducibility as well as specificity to define the kits most acceptable for testing customers' sera, and providing the most reliable information.

A universal one-step real-time RT-PCR for detection of all avian orthoreovirus genotypes

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Newly emerging avian orthoreovirus (ARV) variants have been continuously detected in Pennsylvania poultry since 2011. In this paper, we report our recent diagnostic assay development of one-step real-time RT-PCR (rRT-PCR) for the rapid and universal detection of all ARV reference strains and six σC genotypes of the newly emerging field variants in Pennsylvania (PA). The detection limit of this new rRT-PCR for ARV was as low as 10 copies/reaction of viral RNA, and $10^{0.50}$-$10^{0.88}$ tissue culture infectious dose ($TCID_{50}$)/100µL of viruses. This new rRT-PCR detected all six σC genotypes from ARV field variant strains and reference strains tested in this study. There were no cross-reactions with other avian viruses.

Correlation Between the 1 Day Chick Quality Determined by the Cervantes Test with the First-Week Mortality in the Broilers Farms in the Period September 2014 - February 2015

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The global poultry production has increased at different rates, depending on the characteristics of the economy of each country. The growth of the poultry industry in Venezuela has been sustained and exponential. Is estimated that 48.2% of animal protein consumed in the country is of poultry origin. Given the need of the poultry sector to improve performance, it has understood the impact of chick quality leaving the hatchery on the development of the productive life of broilers. The weight and mortality at 7 days are the most significant parameters in the initial stage. There are different methods to evaluate baby chick quality, including the Cervantes Test, that takes into account the individual physical and microbiological status of 10 baby chicks, allowing qualify chick quality from "excellent" to "not acceptable" on a scale from 100 to 0. The objective of this research was to correlate the 1 day chick quality determined by the Cervantes Test with the first-week mortality in the broilers farms in the period september 2014 - february 2015. A total of 1500 Cobb chicks (150 flocks) were received in the laboratory on the day of his birth, from 7 hatcheries. 10 chicks / flock were individually evaluated by the Cervantes Test, with a physical examination, which includes weight, appearance, eyes, belly button, anus, legs, tarsus, fingers and hydration. Subsequently a microbiological assessment was made, for determination of total bacteria, coliforms,

Staphylococcus aureus, Salmonella sp. and Aspergillus sp. Completed seven days of the birth of the chicks, corresponding to the same flocks in different broilers farms, performance data were reviewed to determine the % mortality of first week. The results were processed by ANOVA, correlation analysis (Pearson) and regression using Statistix 9.0 system. It was shown that exists in 6 of the 7 hatcheries studied, negative linear correlation between the chick quality rating given by Cervantes Test and % mortality of first week, indicating that as this classification increases mortality decreases. We conclude that Cervantes Test is a useful diagnostic tool for estimating the behavior of flocks in early stages, allowing the taking of desitions aimed at improving growth performance and reduce potential economic losses.

Gross and histologic diagnosis of retrograde yolk inhalation in poultry

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Retrograde yolk inhalation (RYI) was diagnosed in commercial laying and broiler breeder hens, backyard hens and quail hens. Clinical presentation ranged from acute death with minimal inflammation to chronic regional granulomatous pneumonia in euthanized poor producing commercial hens. Clinical diagnosis made concurrent with RYI included, “cage layer fatigue syndrome”, concurrent Mycoplasma synoviae infection, “spent layer hens” and ovarian cancer. Necropsy findings for acute death include soft F1 stage vitelline ovarian follicles surround by liquefied yolk, egg yolk peritonitis and air sacculitis with phagocytized yolk. The lungs of hens with acute death often had peripheral congestion and a pale air bronchi pattern. Microscopically, the tertiary bronchi were filled with yolk and had minimal inflammation. The chronic presentation had a firm focal lesion oriented toward the parietal margins of the caudal lungs. Histologically tertiary bronchi and air capillaries were often mineralized and surrounded by fibrosis and chronic granulomatous inflammation. Computerized tomography (CT scan) and latex injection into air sacs were utilized to visualize the anatomical relationship of the ovary to the abdominal air sacs and caudal bronchial ostia. Even though RYI is known to avian pathologists especially in psittacines and more frequently in cockatiels, this common lesion is rarely mentioned in poultry literature and may not be recognized by some veterinarians posting chickens. In combination with several types of ovarian disease, RYI may be an unrecognized component of several syndromes and infectious diseases present in commercial hens.

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The Use of Vaccinated Sentinel Chickens for Isolation of Antigenic Variants of Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease Virus (IBDV) from Delmarva Broilers

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Individually vaccinated broiler chickens held in isolation from day of age until ~35 days of age were placed on a total of 80 Delmarva broiler farms during the Winter and Spring of 2015 and early Winter of 2016. Sentinels were removed at 7 and 10 days post placement on broiler farms, necropsied and tracheal swabbings and bursae of Fabricius collected for virus isolation, RT PCR and virus genotyping. The *in vitro* and *in* vivo characterization of variant IBV and IBDV isolates will be described.

Effect of water electrolyte balance or strong ion difference (SID) on acid-base balance of blroiler chickens

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Electrolyte balance or strong ion difference has been shown to play a major role on blood biochemical values and performance. In practice feeds with the same electrolyte balance can have different effects in different farms. This study was devised to see if water electrolyte balance could have an additive effect on feed electrolyte balance and blood biochemical parameters. Municipal water with an electrolyte balance close to 0 was used as a control and was modified with sodium bicarbonate to increase the electrolyte balance and ammonium chloride to decrease its electrolyte balance. Blood pH, blood gases and electrolytes were measured in each group.(pH, PCO2, HCO3, Na, K, Cl and iCa) Changes in metabolic acidosis or alkalosis can limit birds in the respiratory compensation ability. It is concluded that knowing water composition in stong ions (Na,K and Cl) can help veterinarians improve health and performance of broiler chickens.

Enteric Health

A Comparison of Fungal and Bacterial Populations in Broilers from High and Low Producing Farms.

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Fungus, like bacteria in the past, has been associated with the onset of disease. Little attention has been given to the beneficial effects of fungi with regard to food safety and especially with the gastrointestinal tracts of food-producing animals. In this study, we surveyed the changes that occur in both fungal and microbial populations in health commercial broilers. Four complexes were selected from the southern United States over a 12 month period. For microbiome analysis, crop, duodenum, jejunum and cecal, samples were collected from each bird (n = 5/house) and ten farms per complex on d 30-36. These samples were used for microbiome analysis ($P \leq 0.05$). In the cecal samples collected from the high and low producing farms, no differences were observed between the cecal bacterial populations. However, dramatic

changes were observed in the fungal populations of, and *Eupenidiella* and *Acremonium* geneses in the ceca of birds sampled on the high versus low producing farms. Understanding the fungal and bacterial changes that occur in gastrointestinal tracts of commercial poultry may help us develop a gut health model that will help us target area for potential production and poultry health improvements as well as may be utilized in the development of intervention strategies to control food borne pathogens.

Avian Gut Health 2016 – What's going to happen in the future??

Maarten De Gussem, DVM

VETWORKS, Belgium

Avian intestines will remain the most challenged organ/system in broiler/turkey production in the future. Why is this? The never-ending genetic quest for a bird that grows at the lowest possible feed conversion ratio, thus must grow fast and thus must be hyperphagic. These birds, always eager to consume feed, must always be able to digest and absorb that (relatively) enormous intake of feed. These birds can just cope with that feed intake physiologically when nothing goes wrong. But if we take into account the diverse challenges (physiologic, nutritional and pathologic) that are common under field conditions, almost all birds raised will encounter somewhere in their lives a moment that the physiologic capacities are surpassed by their hyperphagic nature. And it seems that, if we accept the economic and ecologic drivers for further selecting fast growing, efficient birds, we will face this dilemma even more in the future.

This incompatibility between our (societal) needs in raising fast growing birds for meat consumption and the daily reality of facing challenges leads to situations where gut microbiota changes are interacting with anatomic and physiologic changes in the gut. In the past we have mainly focussed on coping with these changes by using antimicrobial growth promoters (AGP) and/or therapeutic antimicrobials to –literally- 'close the gap', but today we have a huge pressure, at least in some areas, to limit or ban those AGP and/or antimicrobials.

Non- Salmonella Associated Cecal Cores In Turkey Poults

Dave Fernandez

AgForte

Cecal cores are typically associated with Salmonella. In response to field observations of the occurrence of cecal cores not associated with Salmonella, trials were conducted with the objective of reproducing the lesions in a controlled environment and determining the causative agent(s) as well as associated risk factors. Day-old poults stressed by withholding feed and water demonstrated a much greater risk of producing cecal cores than control groups that were not stressed. Enterococcus, E. coli, Klebsiella and Clostridium sp. were isolated from the ceca in both the core and non-core forming groups.

Effect of Dietary Vitamin E on *Eimeria tenella*-Induced Oxidative stress in Broiler Chickens.

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An experiment was carried out to investigate the impact of high doses of dietary vitamin E on antioxidant status in broiler chickens (Ross 308) experimentally infected with *Eimeria tenella.* One day old chicks were assigned to five groups (25 each) and given basal diet (A and B) or basal diet supplemented with 100, 316 or 562 mg/kg of vitamin E (C to E), respectively. On the 21st day, all chicks except those in group A were inoculated with *E. tenella* and monitored for any change in blood vitamin E, malondialdehyde (MDA) and superoxide dismutase (SOD). Plasma vitamin E decreased by infection, but increased with dietary vitamin E ($p<0.05$). A significant rise of plasma and erythrocyte MDA was observed in infected birds ($p<0.05$), however, the chicks fed diet with 316 mg/kg added vitamin E had a lower MDA compared to infected controls ($p<0.001$). The erythrocyte SOD was not affected by infection ($p>0.05$), but it was significantly higher in group D than in groups B and E ($p<0.05$). In conclusion, addition of dietary vitamin E at 316 mg/kg can afford antioxidant protection to chickens infected with *E. tenella*, but at higher doses it may aggravate the unbalanced oxidant/antioxidant status.

Chicken astrovirus is associated with enteric disease in experimentally infected chickens

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Enteric diseases are important factors in poultry health. Chicken astrovírus (CAstV) is related to enteric disorders such as runting-stunting syndrome (RSS). In Brazil, CAstV has been detected in chickens showing diarrhea and RSS. The goals of the present work were to determine if CAstV isolated in Brazil could cause enteric disease and to characterize the clinical signs and pathological injuries in the enteric tract of SPF chickens experimentally infected with CAstV. One-day-old SPF chickens were orally infected with an isolated strain of CAstV and maintained for 42 days. Five chickens were collected and slaughtered every seven days, and the duodenum, jejunum and ileum were collected and subjected to molecular, pathological and histopathological examination. Initially, infected chickens showed signs of enteric disease such as diarrhea, ruffled feathers, cloacal pasting as early as one hour after inoculation, and the symptoms evolved to RSS within a few days post challenge. CAstV RNA was amplified and detected by PCR in the three segments of intestine at each harvest time point. The post-mortem examination showed that all segments of the gut were filled with gas bubbles and contained watery feces and undigested food. Histopathological analysis showed significant mild enteritis ($p<0.05$) with an infiltrate of lymphocytes and plasma cells. Increased numbers of intraepithelial lymphocytes and

mitotic cells in the Lieberkuhn crypts were also observed. The mock group did not show any clinical symptoms or pathological injuries. The present work showed that CAstV isolated in Brazil resulted in enteric disease within a few hours of inoculation and caused enteritis, and we also showed that the virus could be present for 42 days.

Food Safety

The Quebec SE committee, a successful industry - governmental agencies – academia collaboration

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Following a 1996 *Salmonella* Enteritidis (SE) outbreak, the Quebec Egg Board decided to establish a zero tolerance SE surveillance program in 1997. The program core includes strict compliance to a code of practice, regular samplings at all levels (breeders, hatchery, replacement pullets and layers) and audits. A strong partnership with the federal and provincial Ministries of Agriculture and Health, and the Faculty of Veterinary Medicine of the University of Montreal, has developed over the years and timely emergency response and recovery plans have been tested and proven. In case of a positive environmental SE sample, results are immediately and automatically cascaded through the communication chain, series of measures are taken to ensure that eggs are immediately diverted to pasteurization and the farm retested, while the producer receives the necessary financial and technical supports. All SE positive flocks are depopulated. Over the years, the program has evolved to include a biweekly collection of unsettable incubation eggs from the broiler breeder sector which are sent to pasteurization, as well as a unique traceability system with each single table egg being stamped with a code. Since the implementation of this program, there has been no SE outbreak related to the consumption of local table eggs in the province of Québec. The success of this program resides not only in a strict surveillance system, in the close partnerships that exists between the different stakeholders, and finally in Quebec Egg producers' commitment to offer a safe product.

Evaluation of the Effectiveness of Various Doses of Bacillus subtilis Probiotic with or without Mannan in Prevention of *Salmonella heidelberg* Colonization.

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There have been several significant trace backs of human foodborne illness associated with *Salmonella heidelberg*. One method of reducing the colonization of *Salmonella* in live birds is use of live competitive exclusion cultures/probiotics. In this study 25% of each pen of chicks (4 treatments, 9 replicates/treatment) were challenged at 1 day of age with a *S. heidelberg* nalidixic acid resistant culture. It was found that the higher dose of B. subtilis numerically lowered *S. heidelberg* levels (MPN) in the birds' environment as measured by boot sock swabs at 40 days of age. It was also found that the higher dose of B. subtilis had the greatest numerical reduction in prevalence (41%) vs. untreated controls (51%) in the horizontal exposed broilers' ceca. Both the low and higher dose had a numerical reduction in the

level of *S. heidelberg* in the ceca of those that were positive as measured by the micro MPN method. A singular *Salmonella* control rarely significantly reduces *Salmonella* in a strong challenge model. Overall, the B. subtilis treatment groups were all effective in reducing horizontal spread of *S. heidelberg*.

Salmonella Enteritidis in Shell Eggs Produced by Backyard and Other Small Layer Flocks

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Salmonella Enteritidis is a leading foodborne pathogen in the United States with many outbreaks in humans traced back to shell eggs. Food and Drug Administration (FDA) requires shell egg producers from a farm with more than 3,000 layer chickens to be in compliance with the FDA Final Egg Rule. However, small flocks with <3,000 birds are currently exempted. Eggs produced by these producers are often marketed via direct retail to restaurants, health-food stores, and farmers markets, or sold at on-farm road-side stalls. Objective of this study was to survey the eggs sold by on-farm roadside stalls and at local farmers' markets for *Salmonella* aiming to estimate the prevalence of *Salmonella* in eggs originating from small flocks with less than 3,000 laying hens, and to characterize the isolates, thus facilitating an assessment of public health risk. In this cross-sectional study, two to four dozen eggs from 240 randomly selected selling points (famers' markets or road-side stalls) representing small layer flocks in 67 counties of Pennsylvania were collected from April to September 2015. Internal contents of the eggs and egg shells were cultured separately for *Salmonella* using standard protocols. *Salmonella* recovered were serotyped and any *S*. Enteritidis isolates present were further characterized by phage typing and pulsed-field gel electrophoresis to evaluate their relatedness to human isolates of *S*. Enteritidis. Of the 240 selling points included in the study, eggs from five (2.08%) selling points were positive for *S*. Enteritidis. Eggs sold by one of the positive selling points contained *S*. Enteritidis in egg shells and eggs from other four selling points had S. Enteritidis in internal contents. Two different phage types (PT8 and PT13a) and two different PFGE types (JEGX01.0034 and JEGX01.0004) were found among these five isolates of *S*. Enteritidis. Two isolates each demonstrated PT8/JEGX01.0004 and PT13a/JEGX01.0034 whereas one isolate belonged to PT8/JEGX01.0034 profile. In summary, this study demonstrated a potential health risk posed by shell eggs produced by small commercial layer flocks. Strategies to minimize *S*. Enteritidis contamination of shell eggs from small flocks will be discussed.

Effectiveness assessment of a genetically modified live vaccine in broilers challenged with *Salmonella* Heidelberg

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Salmonellosis are among the major diseases of commercial poultry and its presence in poultry flocks is responsible for economic losses and risks related to public health. Vaccines are an important tool within the integrated control programs for salmonellosis.

The purpose of this test was to assess cross-protection of Poulvac® ST vaccine in the control of Heidelbeg (SH) in experimentally challenged 3 and 21day birds. Eighty birds were housed, identified and separated into four treatments (T1 – vaccinated and challenged at 3 days of age, T2 – unvaccinated and challenged at 3 days of age, T3 – vaccinated and challenged at 21 days of age and T4 – unvaccinated and challenged at 21 days of age). The inoculum was produced from a Brazilian field strain of SH. At the end of the experiment, samples of cecum and liver/spleen were collected for quantitative and qualitative analysis of SH respectively. The analysis of liver/spleen showed that Poulvac® ST significantly reduced the percentage of positivity by SH in the group challenged at 3 days of age, while in the group challenged at 21 days this difference was next considered to be significant ($p = .0533$). On the other hand, there was no statistically significant difference in SH count in the cecum (CFU/g) in the group challenged at 3 days, but for the group challenged at 21 days the SH counts were significantly lower in the vaccinated group compared to the positive control.

Molecular epidemiology of Salmonella heildelberg isolated from chicken and turkeys

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Salmonella Heidelberg isolated from chickens and turkeys in Midwest and else ware in USA were analyzed using the GelCompar II software (Applied Maths) to generate dendrograms that highlighted the similarities among the fingerprints. Similarity and cluster analysis were also performed on all isolates . In addition all the isolates were examined for resistance to antimicrobials using the SENSITITRE plates. For interpreting resistance, the breakpoints adopted by the National Antimicrobial Resistance Monitoring System (NARMS) guidelines, which meet the FDA, CLSI and EUCAST requirements, were followed. Isolates that exhibited resistance to more than one antimicrobial were considered multi-drug resistant (MDR). Cluster analysis showed that most of the PFGE fingerprints of the *Salmonella* isolated from chickens grouped together with 86.6% similarity (cluster A), while the majority of fingerprints of the strains isolated from turkeys clustered with 87.8% similarity (cluster B). Cluster A and Cluster B shared 85.9%, indicating a relatively high similarity between the majority of the turkey and chicken isolates. However, isolates from turkeys were generally more genetically diverse in comparison to those from chickens. The results of this study and its significance will be presented.

Identification of extended-spectrum beta-lactamases (ESBL) in *E. coli* strains isolated from chicken carcasses obtained from processing plant, public markets and supermarkets in Mexico

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One hundred and fifty *Escherichia coli* strains isolated from 10 chicken carcasses from a slaughterhouse, 10 retail chicken meat samples from 3 markets and 10 samples from 3 supermarkets were tested for the presence of extended-spectrum beta-lactamase (ESBL) genes. After being identified as *E. coli* by biochemical tests, the strains were tested for the presence of ESBL genes *bla*$_{CTX}$ and *bla*$_{TEM}$

and the AmpC beta-lactamase gene *bla*$_{CMY}$ by standard PCR. 51.7% of all strains (50% of the strains isolated form the carcasses and 49% of the strains isolated from retail meat; comprised by 42% of those from markets and 60% of those from supermarkets) were positive to the presence of at least one gene. When comparing the genes present in the different meat samples, we could observe a difference (p=0.058) in the presence of *bla*$_{CTX}$ and *bla*$_{CMY}$, being more frequent in the retail meat from supermarkets. Meanwhile, there was no difference (p<0.06) for the gene *bla*$_{TEM}$ among the samples from the three different origins. Overall, it was possible to identify more ESBL genes in the samples collected from supermarkets. After comparing the genes present in the bacteria from chicken meat and carcasses to those reported in previous studies from humans, it became evident there is a difference between the genes found in chicken carcasses isolates and those from humans, which are more similar to the retail meat isolates, arising the question of whether the genes and isolates found in retail meat come from the birds or they are human contaminants.

MLST genotype of *Campylobacte*r spp. isolated from indigenous chickens in Grenada, West Indies

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This study determined whether multilocus sequence types (MLST) of *Campylobacter* from indigenous chickens differed by location. Grenada, a small island in the southeastern Caribbean, is divided in six parishes. A total of 158 *Campylobacter* species were isolated from randomly collected fecal swabs from indigenous chickens of all six parishes. Of these, 93 were speciated using PCR; 35.5% were identified *C. jejuni,* 6.55% *C. coli* and 58.0% were mixture of *C. jejuni* and *C. coli*. Further, 13 C. jejuni, 19 C. coli and 13 mixed cultures were studied by MLST. ST1769 in *C. coli* was the most predominant type (57.14%) followed by ST5491 and ST3839, which all belong to CC828 cluster. In *C. jejuni,* ST353 and ST1769 were the most prevalent sequence types. However, there was no distinct difference within *Campylobacter* spp. based on parishes from which they were isolated. A majority of STs were the same as those reported previously in multiple studies including our group. However, several STs – 894, 3295 and 6494 were identified for the first time indicating that *Campylobacters* are still evolving and that further research should be continued to understand the essential features of evolution at the genomic level.

Characterizing Outer Membrane Proteins of *Salmonella* Enteritidis Expressed in Egg yolk

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Salmonella enterica serotype Enteritidis (SE) is one of the most common causes of foodborne human illnesses in the United States with more than 350 deaths occurring each year. Shell eggs and egg-based products have been identified as the major source of foodborne SE. The Centers for Disease Control and Prevention estimates about one in 20,000 eggs is contaminated with SE. Regardless of antibacterial compounds present in the egg white and egg yolk, SE is

able to survive inside the eggs. Recent work in our laboratory demonstrated that SE grown in egg yolk was more virulent in a mouse model of colitis than SE grown in Luria Bertani (LB) broth. Therefore, the objective of the present study was to identify and characterize the outer membrane protein (OMPs) that might be responsible for this enhanced virulence and/or fitness of SE in egg yolk. Evaluation of OMP profiles of SE grown in egg yolk and LB revealed that the expression of several proteins were higher in egg yolk in comparison to LB broth. These more abundant proteins were further analyzed to identify their role in SE virulence and suitability as vaccine candidates.

Microbiological Profile of Carcasses in Differents Points of the Production Line of a Birds Processing Plant.

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Chicken meat has properties that favor the development of microorganisms and the rearing, hauling and sacrifice conditions are factors that determine the microbiological quality of carcasses. Therefore, the poultry sector and government agencies, are interested in maintaining proper sanitation in poultry processing plants, which affects the microflora of the product, in order to determine storage conditions, life shelf and public health risks of the final product. Some poultry processing operations increase significantly the bacterial pollution, while others reduce the microbial load. This study determined the microbiological profile of chicken carcasses in different parts of the production chain of a poultry processing plant, to see if the process operations effectively reduce the product microflora. A total of 200 carcasses were taken to the processing plant, directly from two points of the production line. Of these, 100 were chickens after evisceration (AE) and the other 100 were taken at the end of the packing line (EPL). For laboratory analysis rinsing method (APHA) was used, and then counts of mesophilic aerobic (MA), total coliforms (TC) and E. coli (EC) were determinated, according to Venezuelan official method (COVENIN) using PETRIFILM plates, incubated at 37 °C for 24 hours. The average results for MA AE was $2{,}45x10^7$ and at EPL $5{,}38x10^4$. For TC and EC in AE point was $6{,}14x10^6$ and $3{,}36x10^6$ respectively and at EPL $4{,}55x10^2$ and $1{,}98x10^2$. The statistical analysis (comparison of means of Duncan) of the averages obtained allow to conclude that the process operations significantly reduce the microbial load, from gutting until the final product, guaranteeing the safety of this product and consumer security.

Immunology

An educational tool to get references of healthy bursa of Fabricius in commercial broilers

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The bursa of Fabricius plays a pivotal role in the early development of a functional B-cell lymphocyte population in chickens. Several pathologic conditions or mismanagement factors can directly affect the bursa weight or aspect in various extents. Necropsy and further analyses are required to field veterinarians to aim at a proper diagnosis. Therefore, there is first a need to set up a databank to educate field practitioners about what a healthy bursa should look alike in current commercial broilers. Birds were kept free from disease and from vaccination for 6

weeks. Macro- (gross) and microscopic (histopathology) appearance, organ weight and size data were weekly recorded. Results (pictures and figures) will be displayed.

Evaluation of Gimax® Solution as an Immunostimulant Agent in Broiler Chickens.

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A field trial was carried out to investigate the impact of HERBAVITA's phytogenic solution–Gimax® (1lit.:1000lit. of drinking water for week one, 1.5lit.: 1000lit. for week two, and 2lit.: 1000lit. from week three to end of the period), as a possible immunostimulant agent, against Newcastle disease virus (NDV) vaccination in broilers. The experimental farm consisted of two poultry houses divided into three equal compartments. Each compartment housed 4000 as hatched Ross308 broiler chickens. First house received Gimax® up to day 49 over 12 hours in each day, based on manufacturer order, and second house was considered as control. Other rearing conditions were same. Ma5+Clon30® vaccine was sprayed at first and fourteen days of age followed by D78® in drinking water at day fourteen. Spray of Clon30® was done at day twenty-two. At day five of age, a killed NDV+Avian Infulenza (H_9N_2 subtype) vaccine (Intervet, Netherland) intra-muscularly immunized all chickens. From day twenty-one up to the end of rearing period, thirty serum samples were taken from each group with a weekly interval manner and tested for NDV HI method. Results showed that at day twenty-one, NDV titers in Gimax® group were higher than control but it was not significantly differed ($p \geq 0.05$). Results from age of twenty-eight onwards, showed a statistically increase of HI values in Gimax® group compared with control ($p \leq 0.05$). With significantly raising of HI antibody titers against NDV vaccination, it could be concluded that Gimax® solution has a potential to be considered as an immunostimulant agent in broiler chickens.

Assessment of in-ovo broiler substrate deposition site at different embryo developmental stages from three breeder flock ages using a differentiated injection system

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The in-ovo substrate deposition site in chicken embryos at different developmental stages of incubation (17.5, 18.5 and 19 days of incubation) from three broiler breeder flock ages (27, 45 and 56 weeks of age) was evaluated under field-like conditions using an in-ovo machine with a dual pressure injection system. A comparative analysis of the in-ovo substrate deposition site was clearly determined by the egg size and the embryo developmental stage. Results of the different substrate deposition sites in the embryo compartments under these variables will be presented.

Infectious Bronchitis Virus

Insertions in the S1 Spike Glycoprotein of GA13-Type Infectious Bronchitis Virus (IBV) Affect Binding to Chicken Tissues.

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Infectious bronchitis virus (IBV) causes an acute, highly contagious, and economically important disease in chickens. Infection with IBV leads to respiratory disease, drops in egg production, and condemnations at the processing plant following secondary infection, and nephropathogenic strains can cause mortality and severe dehydration from renal disease. The S1 protein, the major antigen on the surface of the virus, is involved in binding to host cell receptors and determining cellular tropism. In 2013 a variant IBV emerged in Georgia (GA13) that was genetically related to the more nephropathogenic Pennsylvania IB viruses. Indeed this GA13 virus seemed to have a mixed pathology for respiratory and nephropathogenic infection, but as the virus became more established in the field the pathology switched to almost strictly respiratory. The S1 protein of GA13 differs significantly from that of the Pennsylvania nephropathogenic strains via three insertions at nt 166-171, 176-178, and 190-192. The aim of this study was to investigate whether these insertions play a role in the binding of S1 to respiratory tract and renal tissue. S1 proteins from GA13 (wild-type and deletions) and PA171 (a nephropathogenic Pennsylvania strain) were expressed in cell culture, and protein immunohistochemistry was performed to measure the binding specificity of each S1 protein to trachea and kidney tissues. Results from this study will shed light on the influence of specific amino acids on the binding of the S1 proteins to different tissue types, which may explain the differences in pathogenicity between respiratory and nephropathogenic strains of IBV.

Homologous and heterologous protection against Brazilian BR-I viruses of Infectious Bronchitis

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Recent epidemiological studies demonstrated very high detection rate and wide geographical distribution of Infectious Bronchitis Virus (IBV) belonging to the BR-I genotype in Brazil. BR-I viruses are frequently detected in broiler, breeder and layers flocks that show disturbs related to IB infection. Genetic analysis of S1 gene shows up to 70% of molecular relationship between this predominant genotype and Massachusetts (Mass) viruses. Field and previous experimental studies showed the failure of Mass vaccines to confer full protection against BR-I-type viruses. Because of these findings, a wild virus belonging to BR-I was attenuated and a candidate live vaccine was prepared. In order to determine the protection conferred by Mass and BR-I-type vaccines against BR-I challenge, vaccination-challenge studies were conducted using SPF and commercial chickens. In the animal trials, vaccine viruses were applied at one day of age by ocular and spray routes. For challenge, three viruses from different origin but belonging to BR-I genotype were used. The evaluation was based on clinical signs, ciliar activity damage and challenge virus colonization and shedding. Full protection was conferred by

BR-I attenuated virus against homologous challenge according to all evaluated parameters while low cross protection was obtained with Mass vaccines. The results of the animals trials showed that full protection against BR-I wild viruses is conferred using homologous virus in the vaccination.

Use of 9CFR Part 107.1 (b) to Control a Variant Strain of Nephropathogenic Infectious Bronchitis Virus in Delmarva Broiler Chickens

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In 2011 nephropathogenic IB (NIB) cases occurred in Pennsylvania and Delmarva (DMV) commercial broiler chicken flocks. The disease was sporadically reported in Delmarva broilers in 2011 and 2012. In 2013, the prototype Delmarva variant isolate, DMV/1639/11, was routinely recovered from respiratory cases but without renal involvement. In Fall and Winter of 2014, NIB was commonly reported in Delmarva commercial broiler flocks and virus isolation and S1 gene sequencing identified the etiologic agents as DMV/1639/11 genotype. The number of respiratory and renal cases yielding DMV/1639/11 soared in 2015 to represent approximately 50% of the disease (or IBV) diagnostic cases submitted to the University's Lasher Lab. Currently available commercial vaccines given to broilers offer limited protection against NIB -induced economic impacts. Faced with limited options, a Delmarva broiler company utilized a recent change to the 9CFR Part 107.1 (b) in which products prepared by a person solely for administration to animals owned by that person shall be exempt from the requirement that preparation be pursuant to an unsuspended and unrevoked license. Results will be presented from safety and efficacy trials in which an emergency autogenous live vaccine was developed to protect broilers from a virulent variant strain of NIBV.

Comparison of protection induced by three different types of 793/B like commercial infectious bronchitis virus (IBV) vaccines against challenge with IS-1494/06 like IBV genotype

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Infectious bronchitis virus (IBV) is an economically important virus infecting chickens, causing large losses to the poultry industry globally. The continuous emergence of new IBV genotypes and lack of cross protection among different IBV genotypes have been an important challenge. 1494/06-like IBV genotype is one of main circulating IBV genotype in commercial flocks in the Middle East including Iran. We evaluated three types of 793/B like commercial IBV vaccines (A, B&C; 14 day; ocular) in combination of H120 (1day; ocular) against 1494/06-like IBV genotype (Intranasal, 10 ^4 EID50, 21 days post last vaccination) through cross-protection study on leghorn SPF chickens. Five days post challenge tissue samples from the trachea, lung and kidney were taken to evaluate ciliary activity, histopathological evaluation and quantitative real-time RT-PCR (Q-PCR). Also, the clinical signs score were also recorded after

challenge. The protection that induced by A, B &C vaccine were 69.2%, 68.2% & 75% respectively. None of the vaccines could induce the proper protection (More 80%) against 1494/06-like IBV genotype. The results indicated different types of vaccines from the same genotype shows the different level of efficacy against challenge virus. In addition, different vaccination programs in this study caused different protection from clinical signs, tracheal or kidney lesions and viral load. In conclusion, the use of more effective vaccines and vaccination policy in combating the one of the dominant IBV variant (1494/06-like) is necessary.

Pathogenicity study of a new emerging Iranian infectious bronchitis virus variant (IR-1) in experimentally infected SPF chickens

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Avian infectious bronchitis (IB) is a major cause of economic loss to poultry industry. Virus primarily affects respiratory tract, but strains differ in their tropism for such other target organs as kidneys and alimentary tract. The objective of this study was to estimate the pathogenicity of a new emerging Iranian infectious bronchitis virus variant (IR-1) which circulates in Iranian poultry farms (and may be in regions) and causes severe economic losses. SPF leghorn chickens were inoculated with 10^4 EID50/0.1 mL of the IR-1 IB virus intranasal. Sera, fecal swabs and different tissue samples were collected on 1,3,5,7,14,21,28 days post infection (d.p.i). Clinical signs, gross pathology and histopathological effects (Immunohistochemistry) were recorded. The amount of virus genome was quantified in RNA extracted from different tissue samples (Trachea, lungs, Kidneys, intestine, etc.) using a quantitative real-time RT-PCR assay (Q-PCR). Severe histologic lesions were observed in the trachea and lung while they were milder in the kidney. Viral RNA was detected in all tested tissues from 1 d.p.i to the last day of experiment. The highest titers were measured in the trachea and feces at days 1 and 5 post infection, respectively. According to IHC findings and Q-PCR data, it can be concluded the Iranian IR-1 like isolate has broad tropism for respiratory tract, digestive system and renal tissue that reflects its epitheliotropic nature, but it causes the most severe lesions in respiratory tract. This is the first pathogenicity study of Iranian IR-1 like IBV isolate. Further knowledge of IBV pathogenesis permits to perform more effective prevention practices.

Infectious bronchitis viruses isolated during the 2005-2014 from broiler chicken farms in Peru

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From 2005 to 2014 were analyzed samples of trachea, lung and cecal tonsils, corresponding 800 broilers from 176 farms of the departments of Lima, Ica and Arequipa. The birds had severe respiratory symptoms consistent with infectious bronchitis after the third week of age. Analyses until 2010 were performed by virus isolation and RT-PCR-RFLP, after 2011 by real-time PCR. By RT-PCR-RFLP were obtained 54 positives samples. Were identified the following strains: 50 IBV- Mass and 2 IBV- Conn strains and 2 samples no identified with RFLP. By real-time

PCR were obtained 24 IBV- Mass, 2 IBV-Conn strains and 2 samples no identified by dissociation curve, possibly a new IBV strain is circulating by this time.

Pathogen Associated Molecular Pattern (PAMP) Receptor Expression in Chickens Before and After IBV Challenge

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Infectious Bronchitis Virus (IBV) is an economically important pathogen in the poultry industry. Although vaccines are extensively used to control IBV, infections persist in chicken flocks. One potential contributing factor to the lack of vaccine mediated immune protection may be the immunological immature state of the chicks when vaccinated. The IBV-specific immune response is initiated in the host by activating pathogen associated molecular pattern (PAMP) receptors during virus infection. We hypothesize that PAMP receptors are important in the control of IBV in chickens and are expressed at lower levels in young chicks. Lymphoid tissues were collected from specific pathogen-free white leghorns at 3 weeks and 2 days of age who were challenged with an IBV field strain. Quantitative-Reverse Transcription PCR was performed to measure mRNA expression of PAMP receptors. Ocular IBV challenged birds at 3 weeks of age showed higher MDA5, TLR-3, TLR-7, TLR-15 and TLR-21 expression than their control counterparts. The magnitude and/or kinetics of expression of MDA5, TLR-7, TLR-15 and TLR differed between 3-wk-old and 2-day-old chickens. This observation is consistent with a role of PAMP receptors in generating IBV-specific immunity.

Can Three Strains of Infectious Bronchitis Virus Given Simultaneously Induce Protection in Chickens?

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Infectious bronchitis virus (IBV) plays a major role in respiratory disease in the U.S. Control of IBV depends largely on the effective use of commercially available vaccines. Live vaccines are manufactured to contain one or two different IBV strains given at recommended doses to achieve optimum protection. However, as many as three IBV vaccine strains have been reportedly administered simultaneously in an attempt to provide a broader spectrum of immunity to poultry. The ability of chickens to immunologically respond to each strain has not been established experimentally. A major concern is that viral interference may occur between two or more of the vaccine strains given at the same time via the same route of administration.

This research study will evaluate the efficacy of simultaneously administering up to three different IBV vaccine strains (i.e. Massachusetts, Arkansas, Delaware 072). Real time reverse transcription polymerase chain reaction (rRT-PCR) will be used to assess viral interference between the vaccine strains in the chicken upper respiratory tract. Challenge of immunity and subsequent virus reisolation attempts will determine the efficacy of the vaccinations.

Peptide Nanoparticle-based Vaccine for Infectious Bronchitis Virus

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Infectious bronchitis virus (IBV) causes respiratory disease in poultry as well as affecting avian renal and reproductive systems. Controlling of IBV is mainly based on vaccination program. Current available lived attenuated or killed vaccines have been challenged by their effectiveness due to large numbers of IBV variants and lack of cross-protection. In our studies, to address this issue, we designed novel IBV vaccines by using a highly innovative platform called self-assembled peptide nanoparticle (SAPN). We repetitively present epitopes of spike protein of IBV in a conformational specific manner. Importantly, we also co-present flagellin on our SAPN to make it self-adjuvant. Immunogenicity study is assessed with assays including ELISA, virus neutralization, lymphocyte proliferation, flow cytometry. Vaccine efficacy will be evaluated by challenging with different virulent strains of IBV in future.

Rapid and specific identification of infectious bronchitis virus (IBV) by real time reverse transcriptase-polymerase chain reaction (RT-PCR) with an internal positive control

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Rapid identification of infectious bronchitis virus (IBV) is not only important for control of the disease, but also to rule out other important respiratory diseases including infectious laryngotracheitis, Newcastle disease, and avian influenza. Real time reverse transcriptase-polymerase chain reaction (RT-PCR) is extremely fast but original tests were developed to detect any and all IBV types. Since vaccine control strategies are only effective when designed around the currently circulating IBV types, it is important to not only rapidly detect IBV but to also identify the type of virus causing disease. Previously we reported on a pan-real time RT-PCR test for IBV as well as specific tests for common types found in the field. In this report we add an internal positive control to verify the integrity of the test as well as document its effect on sensitivity and specificity. Attempts to multiplex specific tests will also be presented.

Multi-strain infection by Infectious Bronchitis variant viruses in broiler and breeder flocks in Latin America

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Infectious bronchitis virus (IBV) is a coronavirus that causes a highly contagious upper-respiratory tract disease in chickens and some strains are also nephropathogenic. The virus can also cause significant egg production losses and mortality in commercial layers and breeders. Coronaviruses, including IBV, have high genetic mutation rates. When those mutations occur in the spike gene they can result in the emergence of variant strain viruses or new serotypes that are only partially or not controlled at all by live and inactivated IBV Massachusetts strains-based vaccines.

Three broiler companies in Colombia with several broiler flocks and one breeder flock presenting overt respiratory signs, kidneys' nephritis lesions, late mortality, decreased egg production and decreased fertile egg quality were investigated for IBV infection. Results from Elisa serology and a molecular diagnostic survey confirmed that flocks had been infected by either one or concomitantly by more than one IBV variant strains (Q1 [China], YEM/L 2865-2005 [Yemen] and K46/10 [South Korea]). Details of the diagnostic investigation and control will be discussed.

Assessment of an immune-complex infectious bursal disease vaccine in "low" maternally-immune commercial broiler chicks

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Infectious bursal disease (IBD) is a major virus disease in chickens worldwide. It is due to infectious bursal disease virus (IBDV), a birnavirus. Only one serotype is pathogenic in chickens (serotype 1), however several antigenic types and pathotypes are circulating in various parts of the world. Prevention is mainly done by vaccination. A global trend of the poultry industry is to move towards hatchery vaccination for convenience reasons. Immune-complex IBD vaccine is addressing the needs for hatchery vaccination, consistent immunization, and progressive displacement of field IBD virus by the attenuated vaccinal IBDV strain. Concerns have been raised about the performance of such vaccine type in chicks (or embryos) in so-called "low" maternal immunity flocks at hatch. Field scale trials were implemented in a commercial operation in the Netherlands in three rounds of broilers. Indeed, country-wise routine breeder vaccination does not include any killed IBD vaccine, and the subsequent passive immunity is minimal. Extensive necropsy, serology, and molecular biology tests were done in three successive rounds of commercial broilers after vaccination. It enabled to monitor the onset of virus release from the immune complex, the kinetics of bursa changes, and the antibody response.

Molecular Epidemiologic Study of Infectious Bursal Disease Viruses in Brazilian Poultry Farms under Different Vaccination Programs

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During a nearly 4 year span (from 2011 to first half of 2014), 251 bursal tissue samples were collected from Brazilian commercial broiler flocks (*Gallus gallus*) throughout the country and imprinted to FTA cards. A total of 81 IBDV strains were successfully detected in the FTA cards imprints and submitted for further identification and molecular characterization. Nucleotide and predicted amino acid sequences of the infectious bursal disease virus (IBDV) surface protein VP2 were used to identify strains of the virus and place them into phylogenetic groups. The amino acids across the hypervariable sequence region of VP2 in this study varied, but around half of positive samples were classified as vaccine virus, in addition there were field virus classified as variant isolates and some classical strains including vvIBDV. Sambles were analyzed according to the vaccination program used in the Poultry flock being grouped into: vectored vaccine, antigen-antibody complex vaccine and conventional live vaccine. The genetic profile of the strains currently circulating in the field is dependent on vaccination program. This information helps us gain a better understanding of the current landscape of IBD in Brazil and provides additional scientific data to support the selection of the most effective vaccination strategies, products and practices to prevent disease.

Efficacy Studies of a Variant Infectious Bronchitis Virus Vaccine

Brianna Ford, Wil Solano, Chris Luther, Kaitlyn Stone, Kristi Moore Dorsey

Ceva Animal Health

Variant IBV strains are continually emerging and are breaking through conventional IBV vaccine regimens causing significant morbidity and mortality creating a need for commercially available, safe and efficacious vaccines. Among the variants currently circulating in the USA, GA08 and GA13, both with respiratory tropism, and DMV/1639/11, a nephropathogenic virus originally isolated from the Delmarva Peninsula, are major concerns for chicken producers. Cevac® IBron, a live IB vaccine, is recommended for vaccination of healthy chickens at day of age by coarse spray for aid in prevention of Georgia type bronchitis, and for vaccination of healthy chickens at day of age by coarse spray for aid in reduction of Georgia 13 type bronchitis. Efficacy studies were conducted by vaccinating day of age specific pathogen free chickens that were challenged with a virulent strain of IBV at 28 days post vaccination, and protection was based on virus isolation of tracheal swabs at 5 days post challenge. In the placebo-vaccinated challenged control group, IBV was isolated from greater than 90% of the chickens.

In addition to the efficacy studies conducted to obtain a USDA license, the following four studies were performed as described above. In one study, the vaccine was administered by coarse spray and significant protection ($p < 0.0001$) was observed following challenge with DMV/1639/11. Similarly, in three other independent studies, the vaccine was administered by the oral route through gel droplet delivery, and significant protection ($p <$

0.0001) was observed when challenged with GA08, GA13 or DMV/1639/11 viruses**.**

Chicken melanoma differentiation-associated gene 5-dependent innate immunity bridging adaptive immunity in infectious bursal disease virus infection

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The present study was to determine if chicken (ch) melanoma differentiation-associated gene 5 (MDA5) sensed infectious bursal disease virus (IBDV) infection and initiated chMDA5-dependent innate immunity, followed by activation of adaptive immunity. During IBDV infection in HD11 cells, IBDV titers and RNA loads increased up to $3.4x10^7$ plaque forming unit (PFU)/mL and 1114 ng/µL, respectively, at 24 hours post infection (hpi). IBDV infection in HD11 cells induced significantly upregulated ($p<0.05$) expression levels of chMDA5, interferon-β (IFN-β), dsRNA dependent protein kinase (PKR), 2', 5'-oligoadenylate synthetase (OAS), myxovirus resistance gene (Mx), interleukin-1β (IL-1β), IL-6, IL-8, IL-10, inducible nitric oxide synthase (iNOS) and major histocompatibility complex class I (MHC class I) up to 59, 693, 4, 286, 22, 5, 146, 4, 4, 15 and 4 folds, respectively. ChMDA5-knockdown HD11 cells had significantly higher ($p<0.05$) IBDV RNA loads at 24 hpi and significantly lower ($p<0.05$) expression levels of chMDA5, IFN-β, PKR, OAS, Mx, IL-1β, IL-6, IL-8, IL-12(p40), IL-18, IL-10, iNOS, MHC class I and CD86 at 24 hpi. The results indicated that chMDA5 sensed IBDV infection in chicken macrophages and was associated with initiation of innate immune response, which bridged to activate adaptive immune response and limited IBDV replication.

Laryngotracheitis

Quantitation of infectious laryngotracheitis virus by a combination of virus propagation in cell culture and quantitative real-time PCR

Girish Sarma and Sarah Jamison

Hygieia Biological Laboratories

The infectivity titer (EID_{50}) of chicken embryo-propagated infectious laryngotracheitis virus (ILTV) is normally done in 9-11 day old embryonated eggs. The cell culture grown ILTV is titered in cell monolayers by using $TCID_{50}$ or plaque assay procedures. In virus-infected cell culture plates, detection of viral CPE is greatly facilitated by immunofluorescence technique (IFA). In the absence of IFA, detection of ILTV induced cytopathic effects (CPE) at higher dilutions often becomes difficult. In this study, we attempted to evaluate the infectivity titers of a cell culture grown ILTV in embryonated eggs and compared the titer results with CPE based $TCID_{50}$ and real-time qPCR findings obtained from the samples originated from the $TCID_{50}$ assay plates. Two standard curves were established, the first with a plasmid containing part of the infected cell protein 4 (ICP4) gene and the second from the 10-fold dilutions of ILTV used to inoculate $TCID_{50}$ assay plates. The primer and probe sequences used for this assay code for the ILTV ICP4 protein- a positive regulator of transcription that is necessary for viral growth. Cq values were compared and infected wells were found to be 4-30X higher in target concentration than similar dilutions with no cells to infect. With this method, it was possible to quantitate precisely the viral concentration in the test samples and compare the results with both CPE-based $TCID_{50}$ in cell monolayers and specific lesion-based EID_{50} in embryonated chicken eggs.

Management

The Effects of Setting Hatching Eggs Upside Down on Embryonic Development and Hatchability

Myles Hill, Josh Lockhart, and Robin Gilbert

Elanco Animal Health and George's Inc.

Eggs from a 47 week old breeder flock were set to observe the losses associated with incubation in the upside down position. Three flats were positioned correctly with three positioned with the small end up. Upon hatch, chicks from both control and experimental groups were evaluated for chick quality characteristics and hatch of fertile. Unhatched eggs were evaluated to determine live vs dead, age of death and embryo positions. Results revealed total early dead was significantly lower in upside down eggs, but significantly higher in total late dead. Percent hatch of fertile tended to be lower in upside down eggs. Navel scabs tended to be higher in upside eggs. Cull chick percent was significantly higher in upside down eggs compared to normal position.

Marek's Disease Virus

Characterization of BACrMd5-REV-LTR virus as MD vaccine in commercial meat type chickens: protection and immunosuppression

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BACrMd5-REV-LTR is a recombinant Marek's Disease virus (MDV) that results from insertion of the long terminal repeat (LTR) of reticuloendotheliosis virus (REV) into the genome of the very virulent MDV strain rMd5. It has been shown that BACrMd5-REV-LTR is poorly oncogenic and confers high protection against challenge with MDV in 15X7 chickens. However, it induces severe thymic atrophy in 15X7 chickens lacking maternal antibodies against MDV; and that limits its use as a vaccine. The objective of the present study was to evaluate the use of BACrMd5-REV-LTR as a vaccine in commercial meat type chickens bearing maternal antibodies against MDV. Two experiments were conducted to evaluate the ability of BACrMd5-REV-LTR to protect against MD and to determine if BACrMd5-REV-LTR induces permanent MDV-induced immunosuppression (pMDV-IS). Our results show that subcutaneous administration of BACrMd5-REV-LTR at day of age conferred high protection (protection index 84.2) against an early challenge (1 day) with vv+MDV 648A strain. Furthermore, BACrMd5-REV-LTR did not induce any immunosuppression in this study; neither thymic atrophy nor permanent immunosuppression. pMDV-IS was evaluated using the MDV-IS ILT model that assess the ability of MDV to downregulate CTL responses during latency.

Effect of Marek's disease vaccines on the immune responses on chicken embryos

Ayanna Glaize, Aneg L. Cortes, Arun R. Pandiri, Isabel M. Gimeno

We have recently demonstrated that vaccination at 18 days of embryonation (18ED) with herpesvirus of turkey (HVT) hastened maturation of the chicken embryo immune system. Day-old-chickens that had been vaccinated *in ovo* with HVT mounted a greater immune response against non-related antigens (keyhole limpet hemocyanine) than chickens that were sham-inoculated (vaccine diluent alone). Furthermore, in ovo vaccination with HVT resulted in expansion of various cell phenotypes in the spleen of 1 day-old chickens (i.e. CD45+MHC-I+, CD3+MHC-II+, CD4+CD8-, and CD4-CD8+). By contrast, administration of other vaccine protocols (such as SB-1 or CVI988) did not have the same effect on the spleen cell population at hatch. Likewise, differences among vaccines were observed regarding the ability to increase IFN-γ transcription in the lung. The objective of the present study was to further compare the effect of the three most commonly used MD protocols (HVT, HVT+SB-1, and CVI988) on the chicken embryo immune responses. Transcription of toll like receptors (TLR-3, TLR-7, and TLR-21) as well as interferon receptors (IFN-AR1 and IFN-AR2) were evaluated in the bursa, thymus, spleen and lung of 1 day old chickens that had been vaccinated with HVT, HVT+SB-1, CVI988, or sham inoculated. Our results demonstrate the each vaccine protocol had a unique effect on the transcription of the evaluated genes and it differs among tissues.

Results and practical implications will be discussed.

Induction of unfolded protein response (UPR) by Marek's disease virus (MDV).

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MDV serves as an excellent model to study how herpesvirus lytic replication triggers cellular stress activation and how the virus modifies the malefic consequences of cellular stress (translation attenuation, ERAD and apoptosis) while allowing beneficial effects (chaperone induction) that support viral replication. In a preliminary study, we investigated the induction of ER stress and activation of the unfolded protein response pathways (UPR) during the course of MDV1 (mildly virulent CU-2) lytic replication in CEFs. We observed a lack of induction of UPR signaling until day 4 post-infection, suggesting that UPR signaling was maintained in a repressed state during this initial phase of infection. However by day 5, we observed a significant transcriptional induction of ATF6 (GRP78/BiP) and IRE1 ($XBP_{(S)}$) pathways while PERK (ATF4) pathway was still maintained in a repressive state. Based on the transcriptional responses observed on Day 5 with mildly virulent CU-2, we followed up our investigation with MDV1 pathotypes of higher virulence, RB1B (very virulent), MD5 (very virulent) and TKING (very virulent plus). Among the different MDV pathotypes, the vv+ MDV strain (TK) induced the highest level of UPR gene expression compared to other pathotypes at 5 days post-infection, despite a more limited replication in these cells. UPR induction seen in vvMDV (RB1B and MD5) infection is relatively lower than that of vv+MDV infected cells indicating that UPR pathways might be more tightly regulated by vvMDVs. Furthermore, we also investigated UPR activation in vv+ MDV lymphomas (latency) and found ATF6 and IRE1α pathways appearing to be active.

My current study presents the activation of unfolded protein response (UPR) during Marek's disease virus lytic replication and latent infection. Herpesvirus replication causes significant amounts of cellular ER stress and consequently UPR activation. In addition, we also examined UPR activation during MDV latent infection in vv+ MDV induced spleen lymphomas.

Pathogenesis of Marek's Disease Vaccine in Turkey Embryos

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Turkeys are the natural host of the turkey herpesvirus (HVT). It is known that HVT is widely widespread in turkey populations although the pathogenesis of HVT in turkeys is not fully understood. HVT is the most commonly used vaccine to protect against Marek's disease (MD) in chickens. In recent years several recombinant vaccines using HVT as a vector has been introduced in the market. One of those vaccines (bivalent HVT and Newcastle disease rHVT-ND) is being used in turkeys in common bases. Furthermore, it is possible that in the future other vaccines (i.e. bivalent HVT and avian influenza, rHVT-AI) might be used as well. Since turkeys are commonly infected with wild type HVT it is advisable that rHVT are administered *in ovo*. In previous studies we have demonstrated that MD vaccines can hasten the development of the immune

system in chicken embryos and this effect varies depending on the vaccine used. The objective of this work was to chronologically evaluate replication of rHVT-ND as well as wild type HVT and two other MD vaccines (SB-1 and CVI988) in the turkey embryo. Vaccine replication was evaluated in the bursa of Fabricius, thymus, spleen, and lung at days 1-3 post vaccination by real time PCR. Our results show that all evaluated vaccines replicated in the embryonic tissues as early as 1 day post vaccination. Differences between vaccine replication and vaccine tropism will be discussed.

Mycoplasma

Current Status of *Mycoplasma gallisepticum* after starting an eradication plan in a grandparent breeder flock in Venezuela.

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Laboratorio de Diagnóstico Protinal-Proagro, C.A. Valencia-Venezuela

In Venezuela, *Mycoplasma gallisepticum* (Mg) remains one of the most incident respiratory pathogens in poultry production stage. For this reason, it began two years ago an eradication plan against this organism in a grandparent breeder flock, trying to improve the health of the birds. To start with the goal, the first step was achieved as a screening method the real-time PCR (qPCR) to monitor closely the birds and the presence of the organism, then the biosecurity plans were strictly reinforced (clothing, transit personnel, education plans, disinfection of vehicles) and once the improvements could be observed inside the farm, began to sampled swabs of the cleft palate of birds every three weeks for the detection of Mg, all houses, from day old to 63 weeks taking 24 samples per house (5 swabs per sample / 120 birds) divided by line (maternal and paternal) and sex for two periods 2013-2014 (2304 samples) and 2014-2015 (1632 samples). For the period 2013-2014 the percentage of positivity for Mg was 11%, while for the period 2014-2015 was 5%, which shows significant decrease positive for this organism. Also, the result shows differences between sex and ages. When the eradication plan was beginning it looked like impossible to achieve these numbers shown here, however with the veterinarian, the diagnostic laboratory tool and biosecurity plan effective, can achieve the objectives, looking to improve and provide birds more productive and healthy every day.

Increasing Virulence of a *Mycoplasma synoviae* Outbreak Strain from Northeast Georgia

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University of Georgia

Mycoplasma synoviae is an infectious pathogen of poultry and although subclincal infection is common, virulent strains may result in respiratory signs and/or swollen joints and footpads. *M. synoviae* can be transmitted horizontally and vertically and the poultry industry has established programs to control *M. synoviae* that focus primarily on eradication using MS-negative breeding stock and good biosecurity practices. In recent years (from 2011) there was an unusually high incidence of *M. synoviae* in the poultry-dense region of Northeast Georgia. In this geographical area the genotype designated as “S-56” was identified in approximately 88% of MS infections in commercial broiler breeder flocks. One of the goals of this study was to compare the virulence of *M. synoviae* isolates of the S-56 genotype isolated in 2012 and 2014. The

MS2014 isolate was from a clinical case (complicated with infectious bursal disease (IBDV)) involving elevated mortality, severe bursal atrophy, airsacculitis and lymphocytic pneumonia. Groups of broilers were inoculated with MS2012, MS2014, and IBDV (recovered from the clinical case). The broilers were evaluated at 14 days post *M. synoviae* infection via serology, air sac and foot pad lesion scoring, real time PCR, and histopathology of the trachea, lungs and bursa. Results showed that MS2014 was more virulent than MS2012, and indicate that avirulent *M. synoviae* strains may progress into more virulent phenotypes after circulating in the poultry population. These results emphasize the benefit of eradicating *M. synoviae* regardless of immediate clinical impact.

Newcastle Disease Virus

Vaccine/Challenge Assessment of Current Newcastle disease Vaccination protocols in Endemic Countries.

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Newcastle diseases is the main concern for endemic countries. Hatchery vaccination protocols in day-old chicks provide early priming and protection against several poultry diseases, such as Marek's disease (MD), Infectious Bursal disease (IBD) and Newcastle Disease (ND). *In ovo* application and subcutaneous (SQ) application of HVT vector vaccines is a current trend in the industry. The aim of these work was to assess the efficacy against a lethal challenge of vaccination programs including VAXXITEK HVT-IBD applied *in ovo* or SQ together with Day 1 VG/GA AVINEW strain spray and or/ SQ with live field boosts. Five groups of 40 one-day-old commercial broilers were used. Groups 1 and 2 compared the effect of *In Ovo* vs. SQ VAXXITEK HVT-IBD on the NDV hatchery program (AVINEW spray). Group 3 used AVINEW SQ + spray instead of the Killed + spray in group 4. Group 5 remained as unvaccinated/challenged control. All vaccinated groups were boosted at day 7 and 17 and challenge with a lethal dose of a Genotype VII velogenic NDV. Significantly higher ($P<0.05$) ND antibody titers were observed when compared with the unvaccinated controls. The control group died within five days after challenge. The survival rates were within the acceptance criteria for all groups (at least 90%). Groups 1= 92.5% and Group 2= 90%. For the groups without and with killed vaccine 92.5 and 97.5, respectively. The results suggest VAXXITEK HVT-IBD can be use *in ovo* or SQ and the suitability of the vaccination programs included in the trial.

Effect of Field Conditions On the Serological Response to Vaccination Against Newcastle Disease Virus.

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For Newcastle disease (ND) a positive relationship between titers and protection has

been established. Nevertheless, the serological response to vaccination, hence the protection can be negatively affected by flaws in the vaccination process and infectious or not infectious immunosuppressive factors. In order to assess the extent of the effect of commercial rearing conditions on the serological response to the vaccination programs from two commercial operations in Venezuela, serological response for ND was assessed at 21, 28, 35 and 42 days of age in sibling birds reared under commercial vs controlled laboratory conditions. The birds reared at the controlled environment obtained at 42 days ELISA titers of 3508 and 2284 for companies A and B respectively ,these were significantly higher ($P<0.05$) than the 1305 and 805 shown by the field birds. Being a fact that all the birds had the same vaccines and vaccination conditions at the hatchery, the results suggest that the differences are generated at the farm level due to environmental and management immunosuppressive factors and/or suboptimal field boost vaccinations; reinforcing the importance of proper husbandry and management in order to achieve the level of immunity expected from the vaccination programs.

Virology: Other

Unusual Lesions Associated with Outbreaks of Avian Encephalomyelitis in Young Brown Chickens

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Avian encephalomyelitis (AE) is a viral disease of worldwide distribution that affects chickens, turkeys, quail, pigeons and pheasants. The disease is caused by a Picornavirus, in a genus called Tremovirus. AE produces neurological signs of ataxia and paralysis in young chickens and drop in egg production in layers. Macroscopic lesions are typically rare. Histologically, disseminated, non suppurative, encephalitis is observed, with lymphocytic infiltrates in the muscularis of ventricular wall. Pancreatitis and myocarditis have also been documented.

Two outbreaks of AE occurred in approximately 14 day old Brown chickens on the same farm, approximately one year apart. Chicks had a history of neurologic signs, increased morbidity and mortality. Eighty live chicks from flocks of 10,000 and 15,000 birds respectively, were examined. Serology and histopathology was performed on birds at different ages between 13 to 56 days. The majority of chicks were ataxic. Gross lesions were not evident. Microscopic lesions included encephalomyelitis with central chromatolysis in the spinal cord and brain, proventriculitis and ventriculitis. Lymphocytic pancreatitis, myocarditis and myositis was observed.

Unusual lesions characterized by neuritis of peripheral nerves, ganglioneuritis of the myenteric plexus of the esophagus, proventriculus, ventriculus and spinal ganglia. Iridocyclitis, choroiditis, and pectenitis otitis media and externa, adrenalitis, splenitis, nephritis and leiomyositis of the intestine and osteomyelitis was also observed. Few chicks had vasculitis of arteries in the muscularis of the ventriculus. AE was confirmed with low titers in young birds that increased in older birds. Outbreaks were related to improper or lack of vaccination in breeder flocks.

Expression of Hexon protein of fowl adenovirus type 4 by utilizing yeast vector

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Hyperpericardium syndrome (HPS) is caused by Fowl adenovirus type 4 (FAdV4) which inflicts mass economic damage in the poultry industry on a world wide scale. The most efficient way to manage HPS up to date was using inactivated vaccine constructed from a whole virus. However, the whole virus vaccines lacked efficacy against variously mutating HPS antigens, which acted as a drawback of whole virus vaccines. Thus, the aim of this study is establishing a system capable of mass producing the Hexon protein, which is a important antigenic determinant in adenoviruses. We introduced the pYEGa-HIR525α vector system based on yeast which is capable of complicated post-modification compared with the widely used E.coli system. Compared from E. coli, the yeast as an eukaryotic organism, is capable of complicated folding of proteins ensuring proper expression of the protein The Hexon coding gene was acquired by gene synthesis and extra sequences such as kozak sequence was added to optimize expression in the yeast and was inserted in to the yeast based pYEGa-HIR525α via infusion method. The expressions of the Hexon protein are to be confirmed by western blotting. The growth of yeast strains containing the gene was confirmed but the experiments to confirm the actual expression of the Hexon protein in yeast and its specificity are currently under way. The formation of specific antibodies will be evaluated by inoculating expressed Hexon protein.

Evaluation of the safety and efficacy of a chicken embryo origin fowl pox virus vaccine in one-day-old broiler chicks

Girish Sarma, Barry A. Kersting and Gary spina

Hygieia Biological Laboratories

Fowl pox is one of the economically important diseases in the poultry industry. Chickens are normally vaccinated when they are 8 weeks of age or older. In high challenge areas, the disease may occur in any age birds. Therefore, under such situation, vaccination is often done as early as 1 day old using cell culture origin fowl pox vaccines. In this study, we evaluated the safety and efficacy of a chicken embryo origin fowl pox virus vaccine administered subcutaneously to 1 day old broiler chicks. Post vaccination mortality and body weight gains were recorded for both vaccinated and unvaccinated control chickens. At 3 weeks post vaccination, all vaccinated and control chickens were challenged against fowl pox to determine the percent protection. The vaccine was found to be safe for subcutaneous administration in

one day old broiler chicks. None of the vaccinated chickens revealed any adverse reactions or mortality associated with the vaccine. Vaccination at day 1 did not significantly affect body weight gain. 100% of the vaccinated chickens were protected against fowl pox virus challenge.

Detection and characterization of two co-infection variant strains of avian orthoreovirus (ARV) in young layer chickens using next-generation sequencing (NGS)

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Using next-generation sequencing (NGS) for full genomic characterization studies of the newly emerging avian orthoreovirus (ARV) field strains isolated in Pennsylvania (PA) poultry, we identified two co-infection ARV variant strains (Reo/PA/Layer/01224a/14, Reo/PA/Layer/01224b/14) from one ARV isolate obtained from the tendons of ARV-affected young layer chickens. Among a total of 842,235 35- to 151-mer sequencing reads, 41,386 reads (4.9%) were identified as ARV sequences. The *de novo* assembly of the ARV reads generated 19 contigs of two different ARV variant strains according to 10 genome segments of each ARV strain, with an average sequencing coverage of 26x to 271x. The two variants shared the same M2 segment. The complete genomes of each of the two variant strains were 23,493 bp in length, and 10 dsRNA segments ranged from 1192 bp (S4) to 3958 bp (L1), encoding 12 viral proteins. Sequence comparison of nucleotide (nt) and amino acid (aa) sequences of all 10 genome segments revealed 58.1-100% and 51.4-100% aa identity between the two variant strains, and 54.3-89.4% and 49.5-98.1% aa identity between the two variants and vaccine strains. Phylogenetic analysis revealed a moderate to significant nt sequence divergence between the two variant and ARV reference strains of chicken, turkey, and waterfowl origin. These findings have demonstrated the first naturally occurring co-infection of two ARV variants in commercial young layer chickens, providing scientific evidence that multiple virus strains can be simultaneously present in one host species. These two ARV variants might easily and quickly undergo subsequent reassortments to generate novel variant strains.

Onsite Surgical Procedures for Backyard Poultry

Annika L. McKillop

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Over the past couple of years, the backyard poultry industry has boomed. There has been a lack of veterinary care in the backyard poultry industry as many veterinarians are not willing, or lack expertise, to see backyard poultry. Many backyard poultry keepers go to online forums and bloggers to get suggestions on how to treat backyard poultry illnesses and injuries. Surgical procedures can easily be preformed onsite at a small poultry farm with the right materials and medications by veterinarians. Successful cases for laceration repair and bumblefoot debridement are presented to educate veterinarians on how to accomplish simple surgeries in backyard poultry.

Wealth of Knowledge

Veterinary Care and Treatment of Urban Poultry

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Chickens have emerged as a companion animal. Backyard poultry owners seek veterinary advice for different health concerns. Management and treatment of pet poultry is different from their commercial counterparts. This paper explores the reasons for submission and veterinary care of backyard poultry from veterinary practices located in large urban areas of Washington State. Poultry presented at the clinic are usually mature chickens, between 1.5 and 2.5 years of age. In the order of most frequent to least common conditions diagnosed at the veterinary practices are egg yolk peritonitis, trauma, egg bound, ascites usually related to carcinoma, sour crop/crop stasis, tenosynovitis, bumblefoot, external parasites, Marek's, Mycoplasmosis, infectious bronchitis virus, coccidiosis, omphalitis, pox, and laryngotracheitis. Serology may not be as relevant for diagnosis as it is for commercial poultry. Also it is surprising that some conditions such as highly pathogenic avian influenza and infectious bursal disease virus have not been seen yet as a problem in urban poultry.

Poultry Respiratory Disease Coordinated Agricultural Project (PRD-CAP)

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Respiratory diseases continue to be a major concern to poultry producers because losses induced by respiratory diseases have significant local and national economic impact to the industry. Protection of poultry by effective prevention and control of diseases is critical to maintain wholesome poultry products, which is the number one animal protein consumed in the United States (US). Such efforts make a significant contribution towards national food security. Our goal is to develop knowledge-based integrated approaches to detect, control and prevent endemic, emerging and re-emerging poultry respiratory diseases in the US. In this project, the efforts of multiple institutions across the country will concentrate on the following four specific objectives: 1) Understand the ecology of poultry respiratory diseases; 2) Investigate the multifactorial etiology involving poultry respiratory diseases; 3) Develop new and improved diagnostic tools, vaccines, and novel preventive measures; and 4) Educate stakeholders for prevention and control of respiratory diseases.

The Development of a Sustainable Commercial Poultry Operation Business in Rwanda

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As a part of the United Nations “Millennial Promise” initiative in 2008, the development of a commercial poultry operation in Sub-Sahara Africa was considered because of the

opportunity to provide needed jobs and affordable protein, as well as to teach business and husbandry skills to a growing population. With the collaboration of multiple business, government and ecumenical partners, the construction of a 4-house commercial layer farm was begun in Musanze Rwanda in the fall of 2009. In May of 2010 the first flock of pullet chicks was placed on the farm. The pullets would be raised up to maturity and would remain in this same house during egg production and then be sold on the market at about 80 weeks of age. Subsequent flocks would be placed in the three other houses approximately every 4 months until the farm was full. This cycle would continue in order to provide for a consistent supply of eggs to the market. After 5 years this operation has had many challenges and issues, but it has succeeded in providing jobs and affordable protein, and is now beginning to consistently sustain profitability as a viable business in Rwanda.

Histomorphometric Evaluations of Hearts from Mature Normal and Cyanotic Roosters with Comparisons to Normal Market-Age Broilers

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We investigated the nuclear concentration of cardiac myocytes in normal roosters and those with transitory cyanosis. The results for mature roosters were also compared to those for normal market-age broilers. The nuclear density of the hearts was determined by enumeration of the numbers of nuclei present within a grid field at 400-x magnification and for 10 consecutive fields for each sample. Most studies focused on the left heart ventricle, but a limited evaluation of the right ventricle was also performed. A dramatic and highly significant reduction in myocardial nuclear density occurred with increased age (average 7-week-old broilers vs average 42-week-old roosters). Although significant differences in the average left ventricle nuclear density could not be shown between normal and cyanotic roosters, a low significance ($p = 0.09$) for the reduction in average nuclear density of the right ventricle was demonstrated for the cyanotic population compared to normal roosters. In addition, if only the data for left ventricle nuclear density below 60/ grid field were considered, there was a dramatic decrease in nuclear density profiles for cyanotic relative to the normal rooster hearts. The histomorphometric findings both document age-associated changes in commercial chickens and provide additional microscopic evidence of cardiac abnormalities (cardiomyopathy) present in both subpopulations of normal roosters and those manifesting with transient cyanosis.

Welfare

Revisiting Animal Welfare Related Production Terms – When to Apply Average, Maximum and Minimum

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When initially implemented over a decade ago, animal welfare programs existed mainly as reactionary responses to audits. In many instances, these programs have evolved into collaborative efforts between customers and suppliers with the common goal of meeting the expectations of the consuming public. Today, auditors take note of production parameters such as culling rate, incidence of lameness,

DOA incidence, and stocking density and there is a general assumption that these figures are directly correlated to the welfare of the animals. Because the outcome (score) of both internal and external audits often have high significance (including ability to conduct business, bonus pay, and continuance of employment), it is crucial that both auditor and producer agree on the appropriate method for calculating these values. Additionally, there is a benefit of tracking and managing appropriate values as measurements of welfare status. Finally, some managers may attempt to manipulate perception by utilizing creative methods to calculate some of these values. The presenter will illustrate the appropriate measurements to be used in calculations as well as the welfare significance of these parameters.