AAAP/AVMA Scientific Program

Nashville, Tennessee
July 2002

AAAP/AVMA ABSTRACT BOOK
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American Association of Avian Pathologists
Schedule of Events
Gaylord Opryland Hotel and Convention Center, Nashville, Tennessee

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8:00 AM-7:00 PM	Board of Directors Meeting *Magnolia Board Room B*
SATURDAY, JULY 13	
8:00 AM-7:00 PM	Board of Directors Meeting *Magnolia Board Room B*
9:00 AM-6:30 PM	ACPV Exam *Cheekwood G,H*
SUNDAY, JULY 14	
7:00 AM-8:00 AM	Awards Committee *Cheekwood E*
7:00 AM-9:00 AM	AAAP/AVMA Liaison Committee *Cheekwood D*
7:30 AM-8:30 AM	Georgia MAM Alumni Group *Presidential D*
7:30 AM-5:30 PM	Poster Session ***Ryman Hall B2***
8:00 AM-5:00 PM	Symposium ***Ryman Hall B3***
9:00 AM-11:00 AM	WVPA Congress Committee *Belmont A*
12:00 PM-1:30 PM	California Alumni Group *Belmont A*
12:15 PM-1:15 PM	Membership Committee *Cheekwood E*
12:30 PM-1:30 PM	Avian Disease Manual Editorial Committee *Cheekwood D*
3:00 PM-7:00 PM	ACPV Board Meeting *Magnolia Board Room A*
5:00 PM-6:30 PM	Long Range Planning Committee *Washington A*
5:00 PM-7:00 PM	Diseases of Poultry Editorial Board *Cheekwood D*
5:30 PM-6:30 PM	Biologics Committee *Magnolia Board Room B*
7:00 PM-10:00 PM	NCSU-CVM Alumni Dinner *Jackson E*
MONDAY, JULY 15	
7:00 AM-8:00 AM	Avian Diseases Editorial Board *Magnolia Ballroom*
7:00 AM-8:00 AM	Preceptorship Committee *Belle Meade B*
7:00 AM-8:00 AM	Toxic, Infectious, Misc. & Emerging Diseases *Belle Meade A*
7:00 AM-8:30 AM	History Committee *Magnolia Board Room A*
7:00 AM-8:30 AM	Foodsafety & Diseases of Public Hlth. Signif. *Jackson C*

7:30 AM-5:30 PM	Poster Session ***Ryman Hall B2***
8:00 AM-5:00 PM	Scientific Program Session A ***Ryman Hall B3***
8:00 AM-5:00 PM	Scientific Program Session B ***Ryman Hall B4***
Noon-2:00 PM	**AAAP Awards Luncheon** ***Magnolia Ballroom***
5:00 PM-6:00 PM	Enteric Diseases Committee *Hermitage E*
5:00 PM-6:00 PM	Tumor Virus Committee *Presidential Board Room B*
6:00 PM-7:00 PM	Animal Welfare and Management Committee *Belmont C*
6:00 PM-7:00 PM	Education Committee *Magnolia Board Room B*
TUESDAY, JULY 16	
7:00 AM-8:00 AM	Biotechnology Committee *Belmont A*
7:00 AM-9:00 AM	ACPV Reception *Magnolia Ballroom*
7:30 AM-5:30 PM	Poster Session ***Ryman Hall B2***
8:00 AM-5:00 PM	Scientific Program Session A ***Ryman Hall B3***
8:00 AM-5:00 PM	Scientific Program Session B ***Ryman Hall B4***
10:30 AM-12:00 noon	**AAAP BUSINESS MEETING** ***Ryman Hall B3***
12:00 PM-1:00 PM	Epidemiology Committee *Belle Mead A*
5:00 PM-6:00 PM	Electronic Information Committee *Belmont B*
5:30 PM-7:00 PM	Respiratory Diseases Committee *Jackson A,B*
WEDNESDAY, JULY 17	
7:00 AM-11:00 AM	Board of Directors Meeting *Magnolia Board Room B*
7:30 AM-Noon	Poster Session ***Ryman Hall B2***
8:00 AM-Noon	Scientific Program Session A ***Ryman Hall B3***
8:00 AM-Noon	Scientific Program Session B ***Ryman Hall B4***

AAAP Symposium on Poultry Vaccines and Vaccination Practices
Sunday July 14, 2002

	I. General vaccinology and vaccine development
8:00	**Vaccination: Protective Antigens and the Protective Immune Response** James Roth
8:30	**Cytokine Responses: What do we Know and Their Effect on Future Vaccine Development** Michael Kogut
8:50	**Vaccine Adjuvant Activity** Virgil Schijns
9:10	**The Performance and Nutritional Cost of Vaccination: Metabolic Changes Associated With an Immune Response** Alfonso Mireles
9:40	**Discussion**
9:55	**Break**
10:15	**Recombinant Vaccines and DNA Vaccines - Unfulfilled Promises** Robert Silva
10:30	***In ovo* Technology - Future Directions** Jagdev Sharma
10:45	**What the End User Needs and Expects from Vaccines** Spangler Klopp
11:00	**Licensing Policy Issues of Poultry Biologics** Tom Mickle
11:15	**Licensing Veterinary Biologics: A Government Perspective** Byron Ripple
11:30	**Discussion**
11:45	**Lunch**
	II. Specific Vaccines and Vaccine Use
	Vaccines Against Respiratory Diseases
1:00	**Infectious Bronchitis: Future Vaccines Against Multiple Serotypes?** Mark Jackwood
1:20	**Infectious Laryngotracheitis Vaccine Development and Use** Calvin Keeler
	Vaccines Against Enteric Diseases
1:50	**Coccidiosis Vaccines: Current Control Issues and Future Control Strategies** David Chapman
2:10	**Vaccines Against Intestinal Viruses of Turkeys** David Halvorson
2:30	**Discussion**
2:40	**Break**
	Vaccines Against Multisystemic Diseases
3:10	**Infectious Bursal Disease Vaccines and Vaccination** Y.M. Saif
3:30	**Live Fowl Cholera Vaccines** John Glisson
3:50	**Discussion**
	Food Safety Vaccines
4:00	**Food safety Vaccines: Salmonella, Campylobacter, Listeria, and Escherichia - Future Approaches** Roy Curtis
4:30	**Wrap-up Discussion** Vergil Davis
5:00	**Adjourn**

2002 AAAP/AVMA SCIENTIFIC PROGRAM

	Monday, July 15, 2002 Session A	Monday, July 15, 2002 Session B
	Moderator: David Swayne	**Moderator: Chuck Hofacre**
8:00	**Comparison Of Neuraminidase Length From Avian Influenza Isolates From Poultry And Wild Birds** Suarez, David	**Effect Of Prior Serial *In Vivo* Passage On The Frequency Of Deposition Of *Salmonella* Enteritidis In Eggs From Experimentally Infected Laying Hens** Gast, Richard and Peter Holt
8:15	**Evolution And Relationship Of Recent H5 Subtype Avian Influenza A Viruses Isolated In North America** Lee, Chang-Won and David Suarez	**Long-Term Characterization Of Bacteriological And Isotype-Specific Antibody Parameters In Chickens With Early *Salmonella Enterica* Serovar *Enteritidis* PT4 Infection** Bautista, Daniel, R. Sheela, Subbiah Elankumaran, Wenxia Song and Robert Heckert
8:30	**Analysis Of The Avian Influenza Matrix Gene Of North American Wild Waterfowl Isolates** Spackman, Erica and David Suarez	**Use Of *sefa* Fimbrial Gene As A Live Recombinant Vaccine Against *Salmonella* Enteritidis In Chickens** Lopes, Vanessa, B Velaydhan, David Halvorson and K Nagaraja
8:45	**Molecular Relationships Between Avian Influenza Virus Subtypes Of H9N2 From Korea And Highly Pathogenic H5N1 From Hong Kong** Kim, Min, Sun Cho, Hyuk Kwon, Eun Lee and Sun Kim	**Induction Of Fimbriae – Specific Antibodies By A Newly Developed Live Salmonella Enteritidis Vaccine In Poultry** Barbour, Elie, Gida Banat, Rabih Talhouk, Faris Jirjis and Mohammad Farran
9:00	**Immunohistochemical (IHC) Staining Of Chorioallantoic Membrane For Evaluation Of Monoclonal Antibodies To Avian Influenza Virus** Weinstock, Daniel, Marlene Castro, Huagang Lu, Iris Wang and Anthony Castro	**Essentials Of A Sustained SE-Risk Reduction Program – Review Of 12 Years Program Implementation** Optiz, H. Michael, Donald Hoenig and Sheila Foster
9:15	**Mucosal Delivery Of Influenza Vaccine Protects Against Lethal Influenza A H5N1 Virus Infection** Tumpey, Terrence and Darrell Kapczynski	**Evaluation Of Salmonella Serogroup Distributions From Commercial Broiler Houses** Ruiz, Jaime, Karen Burns, John Schleifer, Douglas Waltman and Charles Hofacre
	Break 9:30 AM – 10:00AM	**Break 9:30 AM – 10:00AM**
	Moderator: Richard Slemons	**Moderator: Mike Opitz**
10:00	**Application Of A "DIVA" Vaccination Strategy For The Control Of Avian Influenza In Italy** Capua, Ilaria, Giovanni Cattoli, Calogero Terregino and Stefano Marangon	**Identification Of Risk Factors Associated With The Presence Of *Salmonella* In Post-Chill Broilers** Wills, Robert and Hartford Bailey
10:15	**Development And Evaluation Of A Challenge Model For Measuring Efficacy Of Vaccines Against Mildly Pathogenic Avian Influenza Viruses** Swayne, David, Terry Tumpey, Erica Spackman, David Suarez, Joan Beck and Stacey Schultz-Cherry	**Characteization Of *Salmonella* From Poultry Operations** Liljebjelke, Karen, Marie Maier, Tongrui Liu, David White, Charles Hofacre and John Maurer
10:30	**Avian Influenza In Retrospect – A Look At Three Epidemics** Kradel, David	***Campylobacter* Infection Of Young Turkeys** Barnes, H. John, Sophia Kathariou, Jean-Pierre Vaillancourt, Shannon Kozlowicz and Jason Hartsell
10:45	**LPAI H7N2 Experience In Commercial Poultry 2002** Karunakaran, Daniel	**Possible Impact Of Campylobacter Colonization In Commercial Turkeys** Reimers, Nancy, Katy Smith and Sophia Kathariou
11:00	**Laboratory Support For A Recent AI Outbreak In The US** Dennis Senne	**Factors Affecting The Emergence Of Quinolone-Resistant *Campylobacter* In Poultry** Zhang, Qijing and Naidan Luo
11:15	**APHIS Overview of Virginia AI Outbreak** John Hahn	**A Method For Determining Virulence Of *Campylobacter* jejuni.** Reynolds, Don and Sevinc Avinc
	AAAP Awards Luncheon 11:30 AM – 2:00 PM	**AAAP Awards Luncheon 11:30 AM – 2:00 PM**

	Monday, July 15, 2002 *cont'd* Session A	Monday, July 15, 2002 *cont'd* Session B
	Moderator: Louise Dufour-Zavala	**Moderator: Buzz Klopp**
2:00	**Measures Of Pathogenicity For The Pigeon Paramyxovirus Type 1 (PPMV-1)** Panigrahy, Brundaban, Dennis Senne and Janice Pedersen	**Molecular Detection And Differentiation Of Muscovy Duck And Goose Parvoviruses** Pedersen, Janice, Dennis Senne and Brundaban Panigrahy
2:15	**Biological And Molecular Characterization Of Recent Newcastle Disease Virus Isolates Before And After Passage In Chickens** King, Daniel, Glaucia Kommers, Bruce Seal and Corrie Brown	**Hypoglycemia-Spiking Mortality Syndrome: An Update** Davis, James, J. Doman and A. Castro
2:30	**Protection Study Of NDV In Broilers With Two Commercial Vaccines Containing VS-GA And PHY.LMV.42 Strains** Alba, Monica, Eliana Icochea, Rosa Gonzalez and Branco Alva	**Diseases Of Quail Observed In The Alabama State Diagnostic Laboratories: 1996-2001** Hoerr, Frederic, Susan Lockaby, Robert Matthews, Tami Kelly, Francene Van Sambeek and Joel Cline
	Break **2:45 PM – 3:00PM**	**Break** **2:45 PM – 3:30PM**
	Moderator: Pedro Villegas	**Moderator: John Glisson**
3:00	***In vitro* Cultivation Of Pigeon Circovirus** Cardona, Carol and Vanessa Voegtly	**Association Of *Pasteurella Multocida* Transmission Via Artificial Insemination In Turkeys** Morishita, Teresa, Elisabeth Angrick and John Rule
3:15	**Impairment Of Cytotoxic T Lymphocyte Development By Chicken Infectious Anemia Virus Correlates With Increased Viral Load And Altered Cytokine Transcript Levels** Schat, Karel and Carrie Markowski-Grimsrud	**Epidemiology Of Fowl Cholera In Georgia** Zavala, Guillermo, Louise Dufour-Zavala and Douglas Waltman
3:30	**Clinical And Molecular Characterization Of Chicken Anemia Virus Isolates Obtained From Commercial Broiler Farms** Banda, Alejandro, Pedro Villegas, John El-Attrache and Corrie Brown	**Preliminary Evaluation Of A Killed Polyvalent Fowl Cholera Vaccine In Kestrels** Wakenell, Patricia, Bill Ferrier and Brian Evans
3:45	**Chicken Infectious Anemia Virus (CIAV): Detection Of Virus And Antibodies In Commercial Broilers** Sommer, Franz, Carol Cardona and Bruce Charlton	**Histomoniasis In Turkeys – A Reemerging Disease?** Shivaprasad, H. L., G. Senties-Cue, R. Chin, R. Crespo and B. Charlton
4:00	**Summary Of Industry Reovirus Survey** Cummings, Timothy	**Systemic Histomoniasis In Commercial Turkeys** Senties-Cue, Gabriel, H. L. Shivaprasad and Richard Chin
4:15	**Characterization Of Pathogenic Reoviruses Isolated From Broilers And Breeders In The Southeastern United States** Rosenberger, John and Sandra Cloud	**The DOA – A Study In Maximum Inefficiency** Opengart, Kenneth
4:30	**Microscopic Lesions Produced By Recent Isolates Of Infectious Laryngotracheitis Virus From Northeast Georgia In Chickens And Chicken Embryos** Brown, Tom and Mary Pantin	**Assessment Of A Multimedia Turkey Carcass Trimming Training Program** Martinez, Algis, Jean-Pierre Vaillancourt and Eric Gonder
4:45	**Response To Challenge Of Four Michigan Infectious Laryngotracheitis Virus Isolates** Fulton, Richard, Roger Maes and Cunqin Han	**Interpretation Of ELISA Titers For Georgia Broilers, Broiler Breeders And Commercial Layers** Dufour-Zavala, Louise
5:00	**Adjourn**	**Adjourn**
	Tuesday, July 16, 2002 **Session A**	**Tuesday, July 16, 2002** **Session B**
	Moderator: Dick Witter	**Moderator: Rick Sharpton**
8:00	**Marek's Disease Virus In Turkeys: Experimental Infection And A Case Report From France** Miles, Andrea, H. John Barnes, Jeremy Pittman and Jean-Luc Guerin	**Avian Pneumovirus (APV) RNA Isolated From Seagulls Shows Genetic Homology With RNA From APV Isolates From Domestic Turkeys** Velayudhan, Binu, Vanessa Lopes, David Halvorson and Kakambi Nagaraja
8:15	**Persistence Of Marek's Disease Virus In Feather Tips And Spleens Of Chickens As Detected By PCR** Spencer, J. Lloyd and Maria Chan	**Effect Of Age Of Turkeys On Susceptibility To Avian Pneumovirus-Induced Disease And Mitogenic Inhibition** Gerbyshak-Szudy, Heather, Hayet Abbassi and Jagdev Sharma

	Tuesday, July 16, 2002 *cont'd.* Session A	Tuesday, July 16, 2002 *cont'd.* Session B
8:30	**Examination Of Pathogenicity And Vertical Transmission Of Herpesvirus In Japanese Quail** Link, Donald, Patricia Wakenell and Karel Schat	**Development And Evaluation Of A Competitive ELISA For The Detection Of Avian Pneumovirus Antibodies In Various Avian Species** Turpin, Elizabeth and David Swayne
8:45	**Mutagenesis And *In vivo* Studies Of Marek's Disease Virus Encoded Interleukine-8 Like Gene (Vil8)** Cui, Xiaoping, Lucy Lee, Isabel Gimeno, Willie Reed and Sanjay Reddy	**Role Of Local Cellular Immunity In Protection Against Respiratory Challenge With Avian Pneumovirus** Chary, Parag, Mathur Kannan and Jagdev Sharma
9:00	**Role Of 1.8 Kb Mrna Family Of Transcripts in Marek's Disease Virus Pathogenesis** Silva, Robert, Blanca Lupiani and Sanjay Reddy	**Avian Pneumovirus Disease (APV) Outbreaks In Turkeys In The Midwest: A Field Prospective** Medina,Hugo
9:15		**Evaluation Of A Wild Bird Avian Pneomovirus Isolate As A Vaccine Candidate** Halvorson, David, Richard Bennett, Moses Njenga and Kakambi Nagaraja
	Break **9:30 AM – 9:45 AM**	**Break** **9:30 AM – 9:45 AM**
	Moderator: Carol Cardona	
9:45	**History Of Biological Control Of Poultry Diseases In The U.S.** Hitchner, Stephen	
	Business Meeting **10:30 AM – 12:00 Noon** **Lunch** **12:00 Noon – 1:15 PM**	**Business Meeting** **10:30 AM – 12:00 Noon** **Lunch** **12:00 Noon – 1:15 PM**
	Moderator: Lloyd Spencer	**Moderator: Fred Hoerr**
1:15	**Immunologic Tolerance In Chickens Hatching From Eggs Injected With Cell-Associated Turkey Herpesvirus (HVT)** Sharma, Jagdev and Yugen Zhang	**A Comparison Of Diagnostic Tests Performed On Brooding Age Poults And Designed To Identify PEMS Agents** Kozlowicz, Shannon, Jean-Pierre Vaillancourt and H. John Barnes
1:30	**Immunological Features Of The Serotype 1 Marek's Disease Virus Vaccines** Gimeno, Isabel, Richard Witter, Henry Hunt and Willie Reed	**Brooding Performances In 43 Turkey Flocks Depending On Diagnostic Results From Tests Developed For PEMS Related Agents** Vaillancourt, Jean-Pierre, John Barnes, Stacey Schultz-Cherry, Mo Saif, Tom Hooper, Jim Guy, Shannon Kozlowicz, Jason Hartsell, Nancy Reimers, Marion Garcia and David Rives
1:45	**Influence Of B-Haptotype On The Efficacy Of Recombinant Fowlpox Vaccine Protection Against Marek's Disease** Lee, Lucy, Larry Bacon and Richard Witter	**Use Of A Human Cell Line To Induce Helper Virus-Free Replication Of Avian Adeno Associated Virus** Estevez, Carlos, Pedro Villegas and John El-Attrache
2:00	**Development Of New Attenuated Serotype 1 Vaccines With Improved Efficacy Against Marek's Disease Through Cell Culture Passage, Chicken Backpassage, And Retroviral Insertion** Witter, Richard	**Stimulation Of The Innate Immune System Of Broilers With DNA Oligonucleotides Containing CpG Motifs (CpG DNA)** Gomis, Susantha, Dale Godson, Lorne Babiuk, Brenda Allan, Rolf Hecker and Andrew Potter
2:15	**Localization And Profiling Of Reticuloendotheliosis Virus (REV) Protein/S In Cultures Infected With REV Integrated Fowlpox Virus (Rev-FPV) Strains** Tadese, Theodros and Willie Reed	**Dactylariasis In Broiler Chickens** Van Sambeek, Francene, Susan Lockaby and Burton Maxfield
	Break **2:30 PM – 3:00 PM**	**Break** **2:30 PM – 3:00 PM**
	Moderator: Tom Brown	**Moderator: Stan Kleven**
3:00	**Molecular And Biological Characterization Of Naturally Occurring Recombinant Avian Leukosis (ALV) Isolated From Egg-Type Chickens Suffering From Myeloid Leukosis** Lupiani, Blanca, Arun Pandiri, Henry Hunt, Willie Reed and Aly Fadly	***Mycoplasma gallisepticum* In Commercial Layers In Pennsylvania** Davison, Sherrill, Stanley Kleven, Eric Gingerich and Robert Eckroade

	Tuesday, July 16, 2002 *cont'd.* Session A	Tuesday, July 16, 2002 *cont'd.* Session B
3:15	**Quantitation Of Viral RNA And Proviral DNA Levels Of Subgroup J Avian Leukosis Virus (ALV J) By Real Time RT-PCR And PCR** Pandiri, Arun, Blanca Lupiani, Willie Reed and Aly Fadly	**Presence Of Pathogenic Mycoplasmas In Free Flying Visitor Birds From Commercial Layer Farms In The South Peruvian Coastal Area (Chincha)** Noe, Norma, Eliana Icochea, Monica Alba, Rosa Gonzalez, Mirtha Roque and Sharon Levisohn
3:30	**The Design And Evaluation Of Antisense Oligomers As Potential Inhibitors Of Avian Leukosis Virus Replication** El-Attrache, John, Pedro Villegas, Scott Callison and Alejandro Banda	**Diagnosis Of Mycoplasma Infections In Colorado (1999 To Present)** Wooming, Brian, David Ley and Sile Huyan
3:45	**Studies Of Subgroup J Avian Leukosis Virus Infection And Tumors In A Naturally Infected Commercial Broiler Breeder Flock** Dybing, Jody, Arun Pandiri, Larry Bacon and Aly Fadly	**A House Finch-Like *Mycoplasma gallisepticum* Outbreak In A Midwest Turkey Company** Hermes, David and Michael Kopp
4:00	**Response Of Chickens From Three Commercial Broiler Breeders And Two Experimental Lines To Infection With A Field Strain Of Subgroup J Avian Leukosis Virus** Fadly, Aly, Jody Dybing and Arun Pandiri	**The Characterization Of A Naturally Occurring *Mycoplasma gallisepticum* House Finch-Like Strain From Commercial Turkeys** Ferguson, Naola, David Hermes, Victoria Leiting and Stanley Kleven
4:15	**Effects Of Injecting ALV-J Antiserum In Embryonating Broiler Eggs On Localization Of ALV-J In The Tissues Of Broiler Chickens Infected *In ovo*** Gharaibeh, Saad and Tom Brown	**A Preliminary Investigation Of A *Mycoplasma gallisepticum* House Finch-Like Strain As A Vaccine In Turkeys** Ferguson, Naola, Victoria Leiting and Stanley Kleven
4:30	**Incidence Of Cancer In Layer Hens And Effects On Egg Production** Alban-Martinez, Gina, Donna Carver and H. John Barnes	**Sequencing An iss-Containing Fragment From A Conjugative R Plasmid Of An Avian *Escherichia coli* Isolate** Johnson, Timothy, Shelley Horne, Catherine Giddings and Lisa Nolan
4:45	**Mortality Patterns And Etiologies In Geriatric Laying Hens** Carver, Donna, Gina Alban-Martinez and H. John Barnes	**Production Of Monoclonal Antibodies Against Avian *Escherichia coli* Iss** Lynne, Aaron, Shelley Horne, Steven Foley and Lisa Nolan
5:00	Adjourn	Adjourn
	Wednesday, July 17, 2002 **Session A**	**Wednesday, July 17, 2002** **Session B**
	Moderator: Mo Saif	**Moderator: Teresa Morishita**
8:00	**Adaptation Of Virulent Serotype I Infectious Bursal Disease Virus To Avian Macrophages** Abbassi, Hayet, Heather Gerbyshak-Szudy, Hung Yeh and Jagdev Sharma	**Chick Length Uniformity Profiles As A Field Measurement Of Chick Quality?** Hill, Donna
8:15	**A Possible New Serotype 1 Variant Of Infectious Bursal Disease Virus From Korea** Kim, Sun, Hyuk Kwon, Sun Cho, Eun Lee and Min Kim	**Innovations Of In Ovo Technology** Quiroz, Marco, Rafael Correa, William Samson and Erich Bevensee
8:30	**Identification Of A Variant IBDV Strain That Behaves Clinically As A Classic Using Histopathology, PCR, And Monoclonal Antibodies In Commercial Broilers In Peru** Contreras, Manual, Luis Alzamora, Stephanie Mengel Whereat and Fernandez Rafael	**Comparison Of In Ovo And Post-Hatch Injection Of Gentamicin Sulfate In Turkey Poults** Rives, David
8:45	**Mild, Intermediate And Virulent Strains Of Infectious Bursal Disease Virus Differ In Their Ability To Modulate The Immune System** Rautenschlein, Silke, Huang-Yueh Yeh and Jagdev Sharma	**Experiences Raising Commercial Chickens Without The Use Of Conventional Antimicrobial Interventions** Klopp, Spangler
9:00	**The Effect Of Subclinical Infectious Bursal Disease Virus On Iron, Copper, And Zinc Levels In Organs During The Acute Phase Response** Blackmore, Craig, Patricia Wakenell, Kirk Klasing and Donald Link	**Drug Free Chemical Free Broiler Production – Is It Commercially Feasible?** Bahl, Arun and Nino Sorgente

	Wednesday, July 17, 2002 *cont'd.* Session A	Wednesday, July 17, 2002 *cont'd.* Session B
9:15	**Investigation Of Sub-Clinical Infectious Bursal Disease In Broiler Farms With A History Of Production And Disease Problems** Tablante, Nathaniel, Daniel Bautista, Fidelis Hegngi, Conrad Pope and Chinta Lamichhane	**The Effects Of Poultry Production Units On The Air Quality Of Local Environments** Davis, Meredith and Teresa Morishita
	Break **9:30 AM – 10:00 AM**	**Break** **9:30 AM – 9:45 AM**
	Moderator: Nathaniel Tablante	**Moderator: Eric Gingerich**
10:00		**Molecular Biological Tools For Routine Diagnosis And Further Characterization Of Avian Poxviruses** Hafez, Hafez, Torge Hoffman and D. Luschow
10:15	**Recombinant, Attenuated IBDV Vaccine Protects Against Classic And Variant Strains** Liu, Meihong, Meggin Brandt, Yi Liu, Gerard Edwards and Vikram Vakharia	**Diagnosis And Characterization Of Avianpox Viruses From Wild Birds** Tripathy, Deoki and T-J Kim
10:30	**DNA Vaccination Against Classical And Variant Infectious Bursal Disease Virus In Chickens** Wu, Ching Ching, Tsang Long Lin, Hua Chen Chang, Ming Klin Hsieh	**A Review Of *Eimeria mivati* – Edgar And Seibold** Fitz-Coy, Steve, Mike Eckman and Robert Brewer
10:45	**Site-Directed Mutagenesis Of *In Vitro* Expressed IBV Spike Glycoproteins In An Avian Cell Line** Wade, Emma, Mark Jackwood and Deborah Hilt	**DNA Vaccination Strategy Against Avian Coccidiosis** Lillehoj, Hyun and Wongi Min
11:00	**Recognition Of A Unique, Widely-Disseminated Infectious Bronchitis Virus Genotype In North America; Implications For Understanding Spread Of The Disease** Gelb, Jr., Jack, Brian Ladman, Peter Woolcock, Frederic Hoerr, Kalen Cookson, Darrell Trampel, Andre Ziegler and Brian Binnington	**A Comparison Of Coccivac-B To An Anticoccidial Program On Pigmentation Of Broiler Chickens** Mathis, Greg and Charles Broussard
11:15	**Infectious Bronchitis Virus – The "Cal 99" Strain** Woolcock, Peter, Sharon Hietala, Liu-Mei Shih and Michael McFarland	**Multifocal Necrotic Duodenitis: A Novel Disease Of Commercial Laying Hens** Wallner-Pendleton, Eva, Eric Gingerich, Robert Norton, Patricia Dunn and David Kradel
11:30	***In-Ovo* Vaccination For IBV Using DNA Vaccine. A Preliminary Study.** Khan, Mazhar, Jaroslaw Fabis and Ted Grishick	**Enteritis Of Clostridial Etiology In Cage Layers With High Fly Population** Dhillon, A. Singh, L. H. Lauerman, Dennis Schaberg, Daina Bandli and Sylvia Johnson
11:45	**Adjourn**	**Adjourn**

POSTER PROGRAM

1. **The Impact Of The 1999-2001 Italian Avian Influenza Epidemic On The Diagnostic Laboratory**
   Capua, Ilaria and Marilena Campisi Lo Schiavo

2. **A Plasmid-Based Reverse Genetics System For Avian Influenza Virus**
   Lee, Change-Won and David Suarez

3. **Safety Of Ketamine – Xylazine Mixture For Anaethesia Of Some Free Living And Caged Birds**
   Nassef, Mahmoud and Salah Mousa

4. **Evaluation Of Growth Enhancement Antibiotics And Possible Alternatives In Broiler Diets**
   Cherry, Tim and Copie Roberts

5. **Tracking The Claim "If There's One Sick Bird The Entire Flock Is Treated"**
   Gonder, Eric

6. **Comparison Of Antimicrobial Susceptibility Patterns In The Same E. Coli From Field Cases Taken From Blood Agar Plates Versus Macconkey Agar Plates**
   Luna, G. Lynne, Danny Magee, Sue Ann Hubbard, C. Reagan Sadler, Tim Cummings and Melissa Thornton

7. **Vertical Transmission Of Campylobacter Species In Commercial Broiler Chickens**
   Idris, Umelaalim, Margie Lee, Susan Sanchez, Charles Hofacre and Harold Barnhart

8. **A Real-Time Quantitative PCR (qPCR)-Based Serum Neutralization Test For Detection And Titration Of Neutralizing Antibodies To Chicken Anemia Virus (CAV)**
   van Santen, Vicky, Bernhard Kaltenboeck, Kenneth Macklin and Robert Norton

9. **Generation Of Infectious Clones Of Chicken Anemia Virus (CAV) Directly From Clinical Specimens By PCR**
   van Santen, Vicky

10. **Pathological Manifestations Of Pasteurella Multocida Infection In Broiler Chickens**
    Aly, Mohammed and Salah Mousa

11. **Dietary Modulation Of Host Protective Immunity Against Avian Coccidiosis**
    Lillehoj, Hyun, Rami Dalloul and John Doerr

12. **Coronavirus In Turkeys; Are They Turkey Coronavirus Or Infectious Bronchitis Virus**
    Jackwood, Mark, Mary Pantin-Jackwood and Deborah Hilt

13. **Complement Resistance, As Determined By Flow Cytometry, Of Avian *Escherichia coli* Isolates**
    Nolan, Lisa, Catherine Giddings and Shelley Horne

14. **Development Of A Multiplex PCR Protocol To Discern Virulent From Avirulent Avian *Escherichia coli***
    Skyberg, Jerod, Shelley Horne, Catherine Giddings, Curt Doetkott, Richard Wooley, Penelope Gibbs and Lisa Nolan

15. **Educating Service Technicians Through Poultry Workshops**
    Hubbard, Sue and Danny Magee

16. **A Web-Based Biosecurity Training Course For The Poultry Industry**
    Tablante, Nathaniel and Mark Varner

17. **Characterization Of Anaerobic Bacteria Associated With Duodenal Ulcers In Commercial Laying Hens**
    Norton, Robert, Eva Wallner-Pendleton, Eric Gingerich, Paul Patterson, David Kradel and Patricia Dunn

18. **The Effects Of *Tetratrichomonas gallinarum* Infection In Turkeys**
    Bermudez, Alex and Yvette Broomhead

19. **Role Of CPD-Photolyase, And Acidic-Type Inclusion Body Protein In Maintaining The Infectivity And Environmental Persistence Of Fowlpox Virus**
Srinivasan, Viswanathan, William Schnitzlein, Tae-Joong Kim and Deoki Tripathy

20. **Genetic Approaches Toward Characterization Of Avian Poxviruses**
Kim, Tae-Joong, William Schnitzlein and Deoki Tripathy

21. **Evaluation Of A Recombinant Vaccine For Protection Against Fowlpox In Chickens**
Singh, Pratik, William Schnitzlein and Deoki Tripathy

22. **Field Evaluation Of A Novel Bivalent Vaccine Against Infectious Bursal Disease (IBD) And Newcastle Disease (ND) By Mixing Viruses And Antibodies Contained In Hyperimmune Egg Yolk**
Mousa, Salah

23. **Identification Of Multiple Genetic Infectious Bursal Disease Virus Populations (Quasispecies) In Commercial Vaccines Following Plaque Purification**
Spalding, Bethany and Daral Jackwood

24. **Genetic Changes Of Infectious Bursal Disease Virus Passaged In Vero Cells**
Kwon, Hyuk Moo and Soo Joung Kim

25. **Proventriculitis In Broiler Chickens: Effects Of Immunosuppression**
Pantin-Jackwood, Mary and Tom Brown

26. **Genetic Variations Of Avian Infectious Bronchitis Virus Isolates In Japan**
Lin, Zhifeng

27. **Comparison Of The Molecular Typing Of Infectious Bronchitis Virus Variants By The Restriction Fragment Length Polymorphism Assay And Phylogenetic Analysis Of S1 Protein Gene**
Li, Lanqing, Michael Luther and Frederic Hoerr

28. **Gene 5 In Turkey Coronavirus Closely Related To That Of Avian Infectious Bronchitis Virus**
Lin, Tsang Long, Ching Ching Wu, Chien Chang Loa, Aydemir Akin, Herbert Thacker, Thomas Bryan and Tom Hooper

29. **Protection And Productive Parameters In Broilers Using Two Infectious Bronchitis Commercial Vaccines**
Icochea, Eliana, Monica Alba, Rocio Turin, Rosa Gonzalez and Branco Alva

30. **Evaluation Of The Possible Immunological Function Of The Chicken Crop (Ingluvies)**
Vaughn, Lara, Peter Holt, Kelly Crowdis, Kun-Ho Seo and Richard Gast

31. **Effect Of Prior Exposure On Immunologic Response And Cellulitis Lesion Formation In UCD 003 Chickens**
Jeffrey, Joan, K. Tonooka, C. Van Worth and E. Walton

32. **Case Report: Impact Of Electrical Wiring On Broiler Breeder Egg Production**
Magee, Danny and Sue Ann Hubbard

33. **Determining The Etiology Of Unusual Tumors In Chickens; A Case Report**
Miles, Andrea, Jeremy Pittman, H. John Barnes, Isabel Gimeno, Aly Fadly and Richard Witter

34. **Lead Contamination Of Eggs**
Trampel, Darrell, Paul Imerman, Thomas Carson, Julie Kinker, Steve Ensley and Richard Evans

35. **Case Report: Vitamin A Deficiency In Bobwhite Quail**
Lockaby, Susan, Frederic Hoerr and Francene Van Sambeek

36. **Modified PCR For Sexing Monomorphic Psittacines**
Lauerman, Lloyd and A. S. Dhillon

37. **Blindness In Broiler Chicks Due To Retinal Dysplasia**
Shivaprasad, H. L.

38. **Comparison Of The Enzyme Linked Immunosorbent Assay For The Detection Of Antibody To Newcastle Disease Between Commercial Flocks And Non-Commercial Chickens Raised In The Backyard Of Poor Families From Argentina**
Buscaglia, Celina, Veronica Prio and Miguel Risso

39. **Development Of An Inactivated Newcastle Disease Virosome Vaccine That Protects Against Challenge From Velogenic Texas GB**
Kapczynski, Darrell and Terry Tumpey

40. **Persistance Of Avian Pneumovirus In Turkeys Post-Exposure And Length Of Immunity**
Nagaraja, Kakambi, Hyun-Jin Shin, Moses Njenga, David Halvorson and Binu Velayuden

41. **Sequential Pathogenesis Of Experimentally Produced Infectious Proventriculitis: Microscopic And Ultrastructural Changes**
Guy, James and John Barnes

42. **Reo-Virus Infections In Broilers In Upper Egypt**
Seif-Edin, Mostafa and Salah Mousa

43. **Sequence Analysis Of The S3 Gene From A Novel Turkey Reovirus**
Sellers, Holly, Stacey Schultz-Cherry, Valrie Simmons, and Darrell R. Kapczynski

44. **Case Report: Reovirus Outbreak In Mississippi**
Magee, Danny, Sue Ann Hubbard, Tim Cummings, Roy Montgomery and William Martin

45. **A Review Of Poultry Diseases Of Diagnostic Findings In North Alabama**
Kelly, Tami

46. **Resistance Pattern Of Salmonella And E. coli Strains Isolated From Poultry**
Mousa, Heba and Salah Mousa

47. **Cellular Immune Responses To *Salmonella enteritidis* Vaccination**
Lillehoj, Hyun, Masashi Okamura, Uma Babu, Richard Raybourne and Robert Heckert

48. **Effect Of Formaldehyde Usage On In-ovo Injected Eggs**
Throne Steinlage, Sara, Jean Sander and Jeanna Wilson

49. **Oxytetracycline Water Soluble Products: Comparison Of Several Characteristics For Various Commercial Products**
Clark, Steven and Bob Kempa

50. **Microbiological Challenges Of Commercial Turkey Flocks And Methods Of Control**
Karunakaran, Daniel and Thomas Rehberger

51. **Live Attenuated And Killed Salmonella Vaccines Cause An Increase In Cell-Mediated Immunity Among Laying Hens**
Babu, Uma, Mashassi Okamura, Dennis Gaines, Michael Myers, Richard Raybourne, Hyun Lillehoj and Robert Heckert

Sunday, July 14, 2002
Symposium on Poultry Vaccines and Vaccination Practices

Vaccination: Protective Antigens and the Protective Immune Response

James A. Roth
Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA

This presentation will provide an overview of the basic immunology of vaccine responses. The nature of the antigen and location in the body lead to differences in antigen processing and presentation to B and T cells. Multiple signals received by T and B cells provide the information needed to produce an optimal immune response for the particular type of pathogen. Vaccines may or may not lead to the correct signals for the immune system to provide protective immunity. With an improved understanding of the nature of protective immune responses and the identity of protective antigens, it should be possible to develop safer, more effective vaccines for those diseases where the current vaccines are not optimal.

Cytokine Responses: What Do We Know and Their Effect On Future Vaccine Development

Michael H. Kogut
USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX 77845
Co-author: Pete Kaiser

Cytokines are soluble, low molecular weight polypeptides and glycopeptides produced by a broad range of immune and nonimmune cell types that have suppressive or enhansive effects on multiple cellular mechanisms. A complex network of cytokines controls both inflammatory responses and specific immune responses to invasive microbes, which were evolved to protect the host from pathogens. As regulators of the initiation and maintenance of host defenses, cytokines ultimately determine the type of response generated and the effector mechanisms generated to mediate resistance. As effector molecules, cytokines are produced transiently and locally to control the amplitude and duration of the response. Likewise, excessive or insufficient production of a cytokine or cytokines may contribute significantly to the pathophysiology of disease. Therefore, cytokines play pivotal, but paradoxical roles in both the regulation of inflammation and immunity. This review will focus on the use of exogenous cytokines against infectious agents in poultry medicine (a) as adjuvants for vaccines and (b) for their ability to stimulate the ontogeny and activation of neonatal host defenses.

Vaccine Adjuvant Activity

Virgil E.J.C. Schijns
Intervet International BV, The Netherlands

Different infectious diseases are eliminated by distinct types of immune responses and, therefore, require different types of vaccines. Non-replicating whole inactivated vaccine antigens or their subunits inherently lack immunogenicity and are highly dependent on adjuvants for induction and programming of adequate adaptive immune responses. In addition, the type of immune response is strongly dependent on the choice of vaccine adjuvant. Many different substances, ranging from breadcrumbs to aluminum salts or oil emulsions, have been identified to exert vaccine adjuvant activity. Despite a demand for better and safer adjuvants little is known about the mechanism of action underlying adjuvant activity. This lack of knowledge hampers the predictability of vaccine performance and safety. In the last decades knowledge on *in vivo* immunological pathways has increased substantially; especially as a result of new technologies such as *in vivo* gene targeting and gene expression profiling. In addition, immunologists have discovered that the innate immune system plays a key role in alerting and instructing adaptive immune responses. The most important, mutually exclusive, immunological concepts likely explaining the activity of various vaccine adjuvants will be discussed. In addition, examples of new adjuvant candidates for chicken vaccine antigens will be presented.

The Performance and Nutritional Cost of Vaccination: Metabolic Changes Associated with an Immune Response

Alfonso Mireles Jr.
Foster Farms, Delhi, CA

The purpose of this presentation is to review relevant immunology principles correlated with bird performance in a commercial setting and to introduce the concept of enhanced resilience to improve practical poultry performance. Vaccination triggers an obligatory inflammatory response (APR). Modulation of the inflammatory response is more critical

than modulation of the acquired immune response. An inflammatory response is dynamic, adaptive, catabolic, and leads to economic losses. Current selection and management practices select for resilience. Metabolic changes associated with vaccination are, at times, not apparent. The febrile response and body composition changes may quantify the APR. An enhanced inflammatory response may be lethal. Bone metabolism appears to be directly involved with an APR. It is speculated calcium and/or phosphorus metabolism plays a down regulatory role during the APR.

Keywords
Acute Phase Response, Nutritional Immunomodulation, Calcium Metabolism, Vaccination Stress and Nutrition.

Recombinant Vaccines and DNA Vaccines: Unfulfilled Promises

Robert F. Silva
USDA, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI

The poultry industry attempts to control infectious diseases through various means, including appropriate farm management, and the genetic breeding of resistant lines. However, the administration of efficacious vaccines has remained the primary means of controlling most pathogens. Unfortunately, the factory farming used today in the poultry industry has increased chicken densities in poultry houses and subsequently increased both the risk of spreading old pathogens and encouraging the emergence of new pathogens. In this review, we will look at how the advent of recombinant DNA technology promised to provide new classes of more efficacious poultry vaccines. While it is not possible in this review to discuss all poultry pathogens and whether recombinant vaccines could improve existing control strategies, we will look at a few select pathogens, their current vaccines, and whether any existing recombinant vaccines offer opportunities for improvement. Although we will not review the technology of how recombinant DNA vaccines are created, we will discuss possible new strategies to develop yet more novel vaccines and again try to predict whether the future of poultry vaccines lies with conventional vaccines or recombinant DNA vaccines.

***In Ovo* Technology – Future Directions**

Jagdev. M. Sharma
Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota
Co-author: Catherine A. Ricks

Within the foreseeable future, multivalent *in ovo* vaccines that would protect against most or all common diseases, and newer recombinant or subunit vaccines with increased safety for the embryo, are likely to become available. Newer egg injection machines would minimize vaccine wastage by restricting vaccine administration only to eggs containing viable embryos of the desired sex. This development should stimulate other segments of the poultry industry to join the broiler industry in the use of the *in ovo* technology. The commercial use of the technology may expand to include non-vaccine related functions such as chick sexing, administration of immune enhancing and growth promoting substances, and *in ovo* feeding.

What the End User Needs and Expects from Vaccines

Spangler Klopp
Townsends, Inc., Georgetown, DE, 19947

Poultry diseases and vaccines are interesting areas of academic endeavor, but in the real world vaccines are used in chicken production as an aid for controlling specific infectious diseases. Bird quality (welfare), health and economics are critical as chicken meat represents a major source of affordable, quality food protein for consumers around the world. Vaccines have played a major role in the ability of the industry to expand, as evidenced by reduced condemnation rates at processing. Even though these improvements cannot be attributed solely to vaccines, they have played a major role in the control of Marek's Disease (leukosis), respiratory disease and general bird health. While effectiveness and safety of vaccines are tantamount to successful vaccination programs, other issues face the users of vaccines today. These include (1) user friendliness of vaccines, (2) relatedness of vaccine strains to current field exposures, (3) regulatory issues pertaining to vaccine licensing and usage, as well as (4) disease occurrence. In this day and age, public awareness, especially of newly evolving technologies, is becoming an even bigger consideration. Economics, including vaccine reactivity and cost, are also critical.

Licensing Policy Issues Of Poultry Biologics

Thomas R. Mickle
Merial Avian Global Enterprise

Two major topics contain many of the concerns of the veterinary biologics industry today. International trade agreements between the United States and Europe have been in negotiations for several years. The objective is to develop one system for the manufacture and quality control analysis of veterinary biologics in order to promote free trade. The European system is based on Good Manufacturing Practices while the US relies on Title 9 Code of Federal Regulations. The difference in the two regulatory systems requires biologics companies to invest additional resources to conduct development programs that comply with both sets of regulations. The focus of the biologics companies is shifting to the development of vaccines that can be applied on a worldwide basis and local or regional diseases tend to be ignored. The time to market for new products has increased over the past decade. The Center for Veterinary Biologics was formed to centralize the regulatory process in one geographic location. The CVB has not been adequately funded to accomplish its mission and keep pace with the changes in the veterinary biologics industry. Their lack of resources effects the time to market for new products and the competitiveness of US animal health products around the world. Additional funding from the Congress or from the biologics industry itself is necessary in order to change the situation.

Licensing Veterinary Biologics: A Government Perspective

Byron Rippke
USDA, Animal and Plant Health Inspection Service, Center for Veterinary Biologics, Ames, IA

The basic mission of the Center for Veterinary Biologics (CVB) is to ensure that pure, safe, potent, and effective biological products are available to the market place. CVB's goals throughout its regulatory process are to have appropriate and adequate standards, to take those standards and uniformly and consistently apply them, and to help the manufacturers provide quality biological products to an industry that needs them. Quality biological products are a shared goal of CVB, the manufacturers, and the industry. To make that goal a reality requires a partnership of each of these groups. CVB encourages manufacturers, and especially the industry, to actively engage in that partnership process.

Infectious Bronchitis: Future Vaccines Against Multiple Serotypes?

Mark W. Jackwood
Department of Avian Medicine, University of Georgia, Athens, GA

To understand the road blocks in the creation of a cross-protective vaccine for infectious bronchitis virus (IBV), it is necessary to understand the characteristics of the virus, the immune response of the bird that leads to protection, and the molecular basis of different IBV serotypes. Although a vaccine against multiple IBV serotypes currently does not exist, it is hoped that one day it will be possible.

Infectious Laryngotracheitis Vaccine Development and Use

Calvin L. Keeler, Jr.
Department of Animal and Food Sciences, University of Delaware, Newark, DE

Infectious laryngotracheitis (ILT) causes an acute upper respiratory infection in chickens. Vaccination for ILT has generally been used only in areas where the disease is endemic, since current vaccines are themselves mildly pathogenic, with a resulting economic "cost". There is justifiable concern over the negative performance (growth, mortality, feed conversion) associated with current ILT vaccines. After reviewing what is known regarding the avian immune response to this virus, this talk will focus on the status of recent developments in the area of ILTV vaccine development.

Coccidiosis Vaccines: Current Control Issues and Future Control Strategies

H.D. Chapman
Department of Poultry Science, University of Arkansas, Fayetteville, AR

A variety of commercial coccidiosis vaccines are currently available and more are likely in the future. These vaccines contain various species of *Eimeria* depending upon the intended use (broiler or breeder) and may be administered by a variety of

methods. Although vaccines have been used for many years in breeder birds, their application for broilers has been limited. In recent years, however, there has been a resurgence of interest in the use of live vaccines and their use for broilers. This presentation will discuss current live coccidiosis vaccine practices for broiler flocks. Future developments are likely to include the introduction of more vaccines, similar to existing products, and improved methods of delivery for existing and new vaccines. The possibility of vaccinating chicks by in-ovo injection has been proposed. Immunization of hens to confer protection of offspring by injection of specific antigens is under investigation and in the future we may expect vaccines based upon recombinant DNA technology to be introduced.

Vaccines Against Intestinal Viruses of Turkeys

David Halvorson
University of Minnesota, St. Paul, MN

Abstract not available.

Infectious Bursal Disease Vaccines and Vaccination

Y.M. Saif
Food Animal Health Research Program ,The Ohio State University, Wooster, OH

Infectious bursal disease (IBD) has become recognized as one of the most economically important diseases of chickens. Currently, live and inactivated vaccines are in use in commercial flocks. In the USA, the inactivated vaccines are used for breeder flocks and they usually contain classic and variant strains. Live viruses currently used are mostly classic strains. A variety of vaccination programs have been used, dependent on the actual or perceived nature and degree of risk of the disease. A variety of engineered vaccines have been described but they are not commercially available, partly because the protection they induce is not equal to that induced by conventional vaccines and that the cost of conventional vaccines is relatively low. *In ovo* vaccination has been described in the literature but it is not used in the field.

Live Fowl Cholera Vaccines

John R. Glisson
Poultry Diagnostic and Research Center, University of Georgia, Athens, GA

Fowl cholera is still a major problem in broiler breeders. The key factor for reducing the incidence of fowl cholera caused by wild *P. multocida* is controlling rodents in the chicken house. Rodents are the primary carrier of the organism and serve as the challenge reservoir. The other commonly encountered form of the disease is chronic fowl cholera produced by live fowl cholera vaccines. Live fowl cholera vaccines are widely used because they provide protection against many serotypes of *Pasteurella multocida*. Greater vaccine virulence, high vaccine titer, and poor vaccine administration are associated with the incidence of vaccine-induced fowl cholera. Male broiler breeders generally appear to be more likely to develop vaccine-induced fowl cholera than females, particularly when poorly managed or when grown on a severe feed restriction program. This presentation will discuss a few of the management procedures a company should adopt to minimize the incidence of live vaccine-induced fowl cholera.

Food Safety Vaccines: *Salmonella, Campylobacter, Listeria* and *Escherichia*--future approaches

Roy Curtiss III, Xin Zhang, Soo-Young Wanda, and Wendy S. Bollen.
Department of Biology, Washington University, St. Louis, MO

A brief introduction will be given describing commercially available killed and live vaccines to control *Salmonella* infection in poultry with information on relative cost, efficacies and limitations. Most of the presentation will describe research progress in the development of live recombinant and non-recombinant attenuated vaccines to control *Salmonella, Campylobacter, Listeria* and *Escherichia coli* infections in poultry. New genetic design of attenuated *Salmonella* vaccines that are likely to induce higher levels of cross-protective immunity to other enteric pathogens than existing vaccines will be described. These strategies are based on enhanced immune responses to the LPS core antigen, outer membrane protein (OMP) antigens and iron-regulated outer membrane protein (IROMP) antigens. Results with experimental vaccines using the *Salmonella* Type III secretion apparatus for delivery of T cell epitopes to protect against *Listeria* infection and recombinant attenuated *Salmonella* expressing *Campylobacter* protective antigens to prevent *Campylobacter* infection will be presented.

Methods of genetic manipulation of attenuated *Salmonella* to afford biological containment to prevent survival of the vaccine after shedding will be described. Lastly, vaccine design in relation to the types of immunity needed to confer protective immunity and inclusion of immunization of breeders as an enhancement to reducing transmission of these enteropathogens to humans will be considered.

Monday, July 15, 2002
Session A

Comparison of Neuraminidase Length from Avian Influenza Isolates from Poultry and Wild Birds

David L. Suarez
Southeast Poultry Research Laboratory, Athens, GA

The neuraminidase protein of influenza viruses is important in promoting viral spread by cleaving sialic acid off of viral proteins helping to prevent self-aggregation of viral particles. The neuraminidase protein can be variable in size, with the most size variation occurring in the stalk region of the protein caused by sequence deletions. Deletions in the stalk reduce enzymatic activity of the protein. Although the protein is less functional, chicken and turkey isolates are more likely to have stalk deletions than wild bird isolates. A comparison of amino acid sequences of wild bird and poultry isolates will be made to help characterize viruses with and without stalk deletions.

Evolution and Relationship of Recent H5 Subtype Avian Influenza A Viruses Isolated in North America

Lee, Chang-Won
Co-author: David Suarez

Three outbreaks of highly pathogenic avian influenza (AI) in North America involved H5 subtype AI viruses. Though the outbreaks were controlled through an intensive eradication effort, isolation of low pathogenic H5 subtype viruses still routinely occurs. Because of the potential for pathogenicity shift in this subtype of viruses, molecular monitoring of this isolates is important. So, we gathered recent H5 subtype viruses of North American origin and the hemagglutinin genes were phylogenetically analyzed. Based on currently available HA1 gene sequences, a divergence of H5 subtypes into the two geographically distinct lineages (U.S. and Mexican lineage) was obvious. Two sublineages (Puebla and Jalisco) in Mexican isolates were previously described and the recent isolates exhibited an increased rate of mutation from those lineages. Several isolates from Guatemala and El Salvador were also included in this study and they showed the close relatedness with recent Mexican isolates. Further analysis, including the analysis of internal genes, will be conducted to elucidate the evolutionary trend and relationship of this subtype viruses.

Analysis of the Avian Influenza Matrix Gene of North American Wild Waterfowl Isolates

Erica Spackman
Southeast Poultry Research Laboratory, Athens, GA
Co-author: David L. Suarez

Wild waterfowl are considered to be the natural hosts for type A influenza. Therefore when the virus is introduced into other hosts, such as mammals or poultry, mutations occur in the genes of the virus as it adapts to and/or as variants are selected for within the new host. In order to better understand what changes occur we have attempted to determine a consensus sequence for the matrix gene of influenza isolated from wild waterfowl. Analysis, including comparison with previously sequenced influenza isolates, was performed on thirty new influenza matrix gene sequences from virus isolated from North American wild waterfowl.

Molecular Relationships between Avian Influenza Virus Subtypes of H9N2 from Korea and Highly Pathogenic H5N1 from Hong Kong

Min-Chul Kim
Co-authors: Sun-Hee Cho, Hyuk-Joon Kwon, Eun-Kyoung Lee and Sun-Joong Kim

Low virulent avian influenza virus (AIV) of the H9N2 subtype was first isolated in Korea in 1996. We sequenced the nucleotides of the conserved regions of the HA, NA, and NP genes of Korean H9N2 AIV isolated in 2000-01 and compared

for homology with the same or different subtypes of AIV. The Korean isolates formed a separate cluster in the phylogenic trees for the HA and NA genes, distinct from the same subtype viruses from other countries including China. However, the local isolates were located between the groups comprised of some of the highly pathogenic H5N1 viruses from Hong Kong in the phylogenic tree for the NP gene.

Immunohistochemical (IHC) Staining of Chorioallantoic Membrane for Evaluation of Monoclonal Antibodies to Avian Influenza Virus (AIV)

Daniel Weinstock
Pennsylvania State University Avian Diagnostic Lab, University Park, PA
Co-authors: Marlene D. Castro, Huagang Lu, Iris Wang, and Anthony E. Castro

Monoclonal antibodies to Avian Influenza Virus (AIV) hemagglutinin (HA) and nucleoprotein (NP) were evaluated for use in immunohistochemical staining of formalin fixed, paraffin embedded tissue. Specificity and sensitivity of monoclonal antibodies to AIV were evaluated using 9-11 day embryonated specific pathogen free eggs inoculated with various avian viruses including multiple subtypes of low pathogenic AIV. Chorioallantoic membranes were harvested, fixed in 10% neutral buffered formalin and processed by routine histologic procedures. Two non-biotin immunohistochemical staining methods, multiple antigen retrieval techniques and effects of tissue processing were evaluated to develop optimal staining procedures for identification of AIV in tissue sections from AIV infected chickens. Results were validated using tissues from chickens experimentally infected with AIV.

Mucosal Delivery of Influenza Vaccine Protects Against Lethal Influenza A H5N1 Virus Infection

Terrence M. Tumpey
Southeast Poultry Research Laboratory, Athens, GA
Co-author: Darrell R. Kapczynski

Vaccines used to counter avian influenza (AI) in commercial poultry are conditionally licensed for parenteral administration and have provided success at preventing disease, but are less effective in protecting against infection and are not cost-effective. Because respiratory pathogens, like AI invade mucosal surfaces, emphasis should be placed on vaccines that induce strong mucosal immunity. This research seeks to examine mucosal vaccine approaches for controlling AI virus infections in poultry. In a series of vaccine experiments, groups of 2 week-old white-leghorn chickens were vaccinated intratracheally (i.t.), once or twice with an inactivated purified whole A/Turkey/Wisconsin/68 (TW/68,H5N9) vaccine. Vaccine efficacy was measured by survival and virus shedding from orapharyngeal swabs following an intranasal challenge with A/Turkey/Onterio/7732/66 (TO/66, H5N9) virus. Preliminary vaccine results have shown that two (i.t.) vaccinations with 20 μg of inactivated TW/68 vaccine protected chickens against lethal challenge. Future experiments will determine if mucosal vaccination provides greater protection than current parenteral vaccines.

Application of a "DIVA" Vaccination Strategy for the Control of Avian Influenza in Italy

Ilaria Capua
National Reference Laboratory for Newcastle Disease and Avian Influenza
Istituto Zooprofilattico delle Venezie, Spain
Co-authors: Giovanni Cattoli, Calogero Terregino and Stefano Marangon

The paper reports of the laboratory data obtained for the development of a "DIVA" (Differentiating Infected from Vaccinated Animals) strategy for the control of avian influenza of the H7N1 subtype in Italy. The strategy is based on the use of an inactivated oil emulsion vaccine containing the same haemagglutinin (H) subtype as the field/challenge virus, but a different neuraminidase (N). The differentiation between vaccinated and naturally infected birds, was achieved through the development of an *"ad hoc"* serological test based on the detection of specific anti- N1 antibodies. The data of the efficacy of the vaccination campaign in the field, which resulted in the lifting of the bans for intra-community trade are also presented.

Development and Evaluation of a Challenge Model for Measuring Efficacy of Vaccines Against Mildly Pathogenic Influenza Viruses

David E. Swayne
Southeast Poultry Research Laboratory, Athens, GA
Co-authors: Terry M. Tumpey, Erica Spackman, David Suarez, Joan R. Beck, and Stacey Schultz-Cherry

Evaluation of vaccine efficacy for mildly pathogenic (MP) avian influenza virus (AIV) is difficult since such viruses usually do not cause illness or death in experimentally infected poultry. In a series of experimental trials, vaccinated chickens were evaluated for morbidity rates, mortality rates, and serologic response. AIV isolated from the oropharynx and cloaca was quantified by RT-PCR and virus isolation/titration. In the first trial, 3 week-old white Leghorn chickens were immunized with inactivated A/chicken/NY/1342-5/94 (H7N2) MP AIV or sterile allantoic fluid in an oil emulsion base. At 5 weeks of age half of the birds received a second immunization and at 6 weeks of age all chickens were challenged with A/chicken/13142-5/94 (H7N2). Serologically, all 1x (5 weeks of age) and 2x (6 weeks of age) AIV vaccinated birds and all challenged birds (8 weeks of age) were positive for antibodies in the agar gel precipitin test. The mean hemagglutination inhibition (HI) titer for 1x vaccinated birds at 5 weeks of age was 1:13 while at 6 weeks of age the mean HI titers were 1:84 and 1:208 for 1x versus 2x vaccinate groups. Following challenge, the 3 of 10 1x vaccinated birds and 2 of 10 2x vaccinated birds had amnestic responses. No clinical signs or mortality occurred in any of the challenge birds either in sham or inactivated vaccine groups. Correlation between reduction in virus and serologic parameters will be presented.

Avian Influenza in Retrospect – A Look at Three Epidemics

David C. Kradel

Epidemiologic, diagnostic, and control findings in the Pennsylvania avian influenza epidemics of 1983-84 (high path H5N2), 1985-86 (low path H5N2) and 1997-98 (non path H7N2) will be reviewed.

Findings suggested
1) the source of the viruses in all epidemics was the live-bird markets, 2) once virus is introduced into an area of high poultry concentration, spread is extremely difficult to stop and is likely aided by depopulation efforts, 3) recovered flocks present no significant risk, and 4) alternative control and eradication procedures using vaccination and controlled marketing need to be considered.

Bioterrorism and potential public health concerns increase the need for vaccine availability.

LPAI H7N2 Experience in Commercial Poultry 2002

Karunakaran, Daniel

Abstract not available

Laboratory Support for a Recent AI Outbreak in the U.S.

Dennis Senne
National Veterinary Services Laboratories, USDA, APHIS, Ames, IA

Abstract not available

APHIS Overview of the Virginia AI Outbreak

John Hahn

Abstract not available

Measures of Pathogenicity for the Pigeon Paramyxovirus Type 1 (PPMV-1)

Brundaban Panigrahy
National Veterinary Services Laboratories, USDA, APHIS, Ames, IA
Co-authors: Dennis A. Senne and Janice C. Pedersen

Twelve hemagglutinating viruses isolated from pigeons and doves suffering from a central nervous disease and high mortality were identified by monoclonal antibodies as pigeon paramyxovirus type 1 (PPMV-1). Each of 12 isolates was evaluated for pathogenicity in susceptible chickens; none was pathogenic. The mean death time (MDT) of chicken embryos was 71 to 121 hours. However, the PPMV-1 had two attributes similar to virulent Newcastle disease virus: 1) it had an intracerebral pathogenicity index (ICPI) of 0.8 to 1.15, and 2) there were multiple basic amino acids at the fusion protein cleavage site.

Biological and Molecular Characterization of Recent Newcastle Disease Virus Isolates Before and After Passage in Chickens

Daniel J. King
Southeast Poultry Research Laboratory, USDA, ARS, Athens, GA
Co-authors: Glaucia D. Kommers, Bruce S. Seal, and Corrie C. Brown

Six Newcastle disease virus (NDV) isolates recovered from chickens, exotic, and wild birds were characterized before and after four passages in specific-pathogen-free 2-week-old white leghorn chickens. Hemagglutination-inhibition assays against a battery of monoclonal antibodies and nucleotide sequence analysis of the fusion protein cleavage site of the isolates were utilized to determine if antigenic or genetic changes had occurred as a result of chicken passage. The results were compared to pathotyping tests previously performed and to the Office International des Epizootes criteria for NDV virulence. Marked virulence increase was observed after passages with one of the six isolates, an isolate from an exotic dove. An isolate from an exotic pheasant was virulent before passage and demonstrated increased virulence after passage. Passage in chickens produced no marked change in virulence of the other four isolates. Although there was antigenic diversity among the isolates there was no antigenic or fusion protein cleavage site change associated with any of the observed changes in virulence of the isolates.

Protection Study of NDV in Broilers with Two Commercial Vaccines Containing VG-GA and PHY.LMV.42 Strains

Monica Alba
School of Veterinary Medicine, University of San Marcos
Co-authors: Eliana Icochea, Rosa Gonzalez and Branco Alva

The protection of vaccinated broilers with two intestinal and one respiratory commercial vaccines were evaluated. Vaccines containing VG-GA, PHY.LMV.42 AND CLONE 30 strains were used in 280 Ross 308 broilers divided in seven groups of 40 birds each. Groups 1, 2, and 3 were vaccinated with one dose of live vaccine at 8 days old, the groups 4 and 5 had two doses of live vaccine at 8 and 18 days of age. Group six had an oil vaccine at one day old and a live vaccine at 8 days of age. Group seven was the unvaccinated control group. All the groups were challenged via eye drop at 28 days of age with a viscerotropic velogenic strain of NDV. Blood samples were collected at 1, 8, 18, 28, 35 and 46 days and tested with ELISA and HI for antibody detection. Mortality, clinical signs, lesions and body weight were recorded. The results allow us to state that all six vaccinated groups were protected of NDV. Groups with the two intestinal strains showed an efficient protection against viscerotropic velogenic Newcastle disease.

***In vitro* Cultivation of Pigeon Circovirus**

Carol J. Cardona
Veterinary Medicine Extension, University of California-Davis
Co-author: Vanessa Voegtly

Pigeon circovirus had been diagnosed in birds with severe immunosuppression. Although it has been implicated as an underlying factor in disease outbreaks, research has been limited by the inability to isolate the virus in vitro. We will present information on two methods that we have developed for virus isolation directly from infected tissues and passage of the virus in the laboratory.

Impairment of Cytotoxic T Lymphocyte Development by Chicken Infectious Anemia Virus Correlates with Increased Viral Load and Altered Cytokine Transcript Levels

Karel A. Schat
Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY
Co-author: Carrie J. Markowski-Grimsrud

Previously we have shown that infection with reticuloendotheliosis virus (REV) and Marek's disease virus results in the generation of specific cytotoxic T lymphocytes (CTL). However, natural exposure to chicken infectious anemia virus (CIAV) apparently abrogated CTL responses unless chicks were positive for CIAV maternal antibodies. To further analyze the influence of CIAV on CTL responses maternal antibody positive and negative chicks were infected with CIAV and REV. REV-specific CTL responses were impaired in antibody negative chicks, but not in antibody positive chicks. Quantitative real-time PCR analysis indicates that the absence of CTL responses correlates with increased viral load and altered cytokine transcript levels.

Clinical and Molecular Characterization of Chicken Anemia Virus Isolates Obtained from Commercial Broilers

Alejandro Banda.
College of Veterinary Medicine, The University of Georgia, Athens, GA
Co-authors: Pedro Villegas, John El-Attrache and Corrie Brown

Five chicken anemia virus (CAV) field isolates obtained from poor performance broiler flocks in Georgia were isolated using SPF chickens. The five isolates caused a severe clinical syndrome in SPF birds inoculated at one day of age. The onset of the clinical signs appeared between 18 and 20 days post inoculation and the signs included depression, ruffled feathers, anorexia and mortality. A decrease in the packed cell volume (15% to 25%) in inoculated birds was observed. At necropsy, inoculated birds exhibited severe bone marrow atrophy as well as severe thymic atrophy, with a 60% decrease in thymic weight in comparison with the uninoculated control group. No differences were observed in splenic and bursal weights. *In situ* hybridization detected replication of the virus mainly in thymic cortex and spleen, with few cells exhibiting viral infection in the bursa. Similarities were observed via nucleotide and amino acid sequence analysis between the five CAV field isolates and the DelRos strain.

Chicken Infectious Anemia Virus (CIAV): Detection of Virus and Antibodies in Commercial Broilers

Franz Sommer
University of California-Davis
· Co-authors: Carol J. Cardona and Bruce R. Charlton

Broiler chickens were sampled weekly, beginning at day 1. Ten birds each were submitted from two houses on different ranches.

CIAV PCR and serologic tests demonstrated that virus and antibodies decreased and disappeared between days 14 and 21. From day 28 on, increasing numbers of PCR and ELISA positive samples were found in both flocks.

Both tests were closely correlated suggesting that broilers are vertically infected with CIAV and also have maternal antibodies. We hypothesize that once maternal antibodies wane, viral replication begins as well as exposure to field viruses.

Flock performance and complete diagnostic testing results will be presented.

Summary of Industry Reovirus Survey

Timothy S. Cummings
College of Veterinary Medicine, Mississippi State University

As chair of the AAAP Epidemiology committee, we will have initiated an industry reovirus survey due to its recent "resurgence". Findings will be summarized and presented.

Characterization of Pathogenic Reoviruses Isolated from Broilers and Breeders in the Southeastern United States

John K. Rosenberger
Department of Animal and Food Science, University of Delaware, Newark, DE
Co-author: Sandra S. Cloud

Reoviruses were isolated from tendons, synovial fluids, livers, bursae of Fabricius and proventriculi taken from young broilers and broiler breeders produced in Alabama, Mississippi, Arkansas and Georgia. Tissues collected were from flocks characterized by increased mortality, uneven growth, poor feed conversions and leg problems (tenosynovitis and inflamed food pads). Reovirus isolates were pathotyped in broilers and SPF leghorns and evaluated antigenically by cross neutralization and *in vivo* challenge. Efficacy of available inactivated and live reovirus vaccines were compared in terms of their ability to induce protection against the contemporary isolates and previously described strains. The safety of several live reovirus vaccines was also assessed.

Microscopic Lesions Produced by Recent Isolates of Infectious Laryngotracheitis Virus from Northeast Georgia in Chickens and Chicken Embryos

Tom P. Brown
Departments of Avian Medicine and Veterinary Pathology, College of Veterinary Medicine, University of Georgia
Co-author: Mary J. Pantin

Classically, infection with Infectious Laryngotracheitis Virus (ILTV) produces acute hemorrhagiconecrotic tracheitis in broiler chickens and multifocal necrotizing chorioallantoitis in inoculated embryos, with formation of numerous epithelial syncytial cells and viral inclusion bodies in both lesions. Recent naturally-occurring cases of ILTV infection in chickens have early and severe diffuse deciliation, epithelial hyperplasia, and severe subepithelial lymphoid infiltrates, but fewer syncytial cells and inclusions. Lesions in embryos are similarly severe with low syncytia/inclusion numbers. These non-classical ILTV lesions in chickens and embryos will be illustrated and followed chronologically using routine histologic techniques as well as immunohistochemical identification of ILTV in tissue sections.

Response to Challenge of Four Michigan Infectious Laryngotracheitis Virus Isolates

Richard M. Fulton
Animal Health Diagnostic Laboratory, Michigan State University
Co-authors: Roger Maes and Cunqin Han

To determine the pathologic characteristics of isolates from recent outbreaks of infectious laryngotracheitis (ILT) in Michigan, 5 groups of 24 routinely (non-ILT) vaccinated pullets were placed in individual isolation rooms. Field isolates were used to infect four groups while a standard challenge strain was used to infect the remaining group. Birds were monitored twice daily for clinical signs. All dead birds and birds surviving infection were necropsied. Gross lesions were recorded and scored. Trachea was formalin fixed, processed and three sections were evaluated microscopically. Each section was given a microscopic lesion score. The results were compared.

Monday, July 15, 2002
Session B

Effect of Prior Serial *in vivo* Passage on the Frequency of Deposition of *Salmonella enteritidis* in Eggs from Experimentally Infected Laying Hens

Richard K. Gast
Southeast Poultry Research Laboratory, Athens, Georgia
Co-authors: Jean Guard-Petter and Peter S. Holt

Experimental infection models are important tools for studying the deposition of *Salmonella enteritidis* inside eggs. Oral inoculation is often employed in such experiments because it is believed to closely simulate naturally occurring *S. enteritidis* infections of chickens, but relatively low egg contamination frequencies have been obtained in many recent oral infection studies. The present study considered whether repeated *in vivo* passage of a phage type 13a *S. enteritidis* strain in chickens would affect its subsequent frequency of deposition in eggs by experimentally infected hens. The incidence of egg

contamination was determined in groups of hens inoculated orally with the original isolate or with derivatives obtained after prior serial passage and re-isolation from tissues of infected hens. Passaged *S. enteritidis* isolates, especially those recovered from reproductive organs, were associated with a higher incidence of egg contamination than the original strain in some trials.

Long-term Characterization of Bacteriological and Isotype-specific Antibody Parameters in Chickens with early *Salmonella enterica* serovar *enteritidis* PT4 Infection

Daniel A. Bautista
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland
Co-authors: R. R. Sheela, Subbiah Elankumaran, W. Song and Robert Heckert

Salmonella enteritidis phage type 4 (SE PT4) in poultry is still a very important public health concern because of its implication in foodborne outbreaks. There are few studies on long term SE immunobiology that follow birds well into the laying period after early SE PT4 infection. In this study, we examined SE shedding patterns and the relative behavior of isotype-specific antibody responses against SE in infected layers up to 32 weeks of age. SPF leghorns (n=256) were separated into 2 groups. One group was infected with 10^5 CFU/ml of SE PT4 at 3d of age and 16 birds from each group were harvested at 2,4,8,12,16,20,24, and 32 wk of age. Cecal carriage of SE PT4 was determined by direct plating decimal dilutions of cecal contents. Percentage recovery of SE over time was done by enrichment of the cecal suspension. Serum was collected and an indirect anti-Salmonella Ig-specific ELISA was performed to determine IgA, IgG, and IgM antibody levels over time. Immunohistochemical localization of SE in the spleen and intestines was done using SE-specific antisera.

The %SE-positive birds at 2, 4, 8 and 12 wk decreased considerably from 75%, 44%, 18% to 0%, respectively. All birds were negative for Salmonella on the succeeding sampling periods, except for wk 16 which had one positive bird. Mean Salmonella counts on direct plating decreased from 10^5 to10^4 to 10^2 CFU/gm cecal content in the first 3 sampling periods. In the spleen, organisms were primarily localized in the macrophages. In the intestines, clumps of intracellular bacteria were localized in the mononuclear cells dispersed in the lamina propria, and crypts up to 8 weeks. The organisms could be localized intracellularly in the epithelial cells of the oviduct and occasionally in the spleen and the gut from 20-32 weeks of age. However, no Salmonella could be isolated from the ceca and oviducts from 20-32 weeks of age. The intestinal and systemic IgA responses were minimal and were not significantly different than the controls. However, Salmonella specific serum IgM antibodies were significantly higher at 16 and 20 weeks of age, while the IgG responses increased from 12 weeks and peaked at 20 weeks of age. The increase in IgM and IgG antibodies in the serum coincided with the clearance of the organisms from the gut.

Use of *sef*A Fimbrial Gene as a Live Recombinant Vaccine Against *Salmonella* Enteritidis in Chickens

Vanessa C. Lopes
University of Minnesota, St. Paul, MN
Co-authors: Binu Velayudham, David A. Halvorson and Kakambi V. Nagaraja

Salmonella Enteritidis is considered an important public health issue. *S.* Enteritidis SEF14 fimbrial protein has been suggested to play a role in the colonization of the host tissue. In this study, the *sef*A gene, responsible for the major subunit of the SEF14 fimbrial protein, was cloned into a temperature-sensitive expression vector and transformed into non-pathogenic chicken *E. coli* cells. These transformed cells were used as a live oral recombinant vaccine, attempting to elicit immune response and protection in chickens against *S.* Enteritidis.

Induction of Fimbriae-Specific Antibodies by a Newly Developed Live *Salmonella* Enteritidis Vaccine in Poultry

Elie K. Barbour
Department of Animal Science, American University of Beirut,
Co-authors: Gida Banat, Rabih Talhouk, Faris Jirjis and Mohammad Farran

Four groups of commercial chicken layers will be included in this study to evaluate the induction of fimbriae-specific antibodies in sera and egg yolk by a newly developed live Salmonella Enteritidis (SE) vaccine. The first group of layers will be administered the newly developed live SE vaccine followed by a challenge with a highly virulent SE, the second group will be administered a killed SE vaccine followed by a challenge, the third group will be given only a challenge, and the fourth group will be unvaccinated and unchallenged. Protection against SE invasion into the visceral organs will be included in this study.

Essentials of a Sustained SE Risk-Reduction Program: Review of 12 Years of Program Implementation

H. M. Opitz
Cooperative Extension /Animal & Veterinary Science Department, University of Maine

The increase of food-borne Salmonella enteritidis (SE) outbreaks in the Northeast and the association of many outbreaks with the consumption of egg-containing dishes prompted the State of New York in 1988 to impose conditions on the import of eggs from certain states. The NECAD Salmonella committee responded by developing of recommendations for a voluntary Model Egg Quality Assurance Program. Aspects of these recommendations formed the basis for the development of State Egg Quality Assurance or SE Risk Reduction programs in Pennsylvania and New England and later for the USDA Traceback Program, Pennsylvania Pilot Program and other programs. The goal of all programs was a) to pinpoint risk flocks b) to implement programs that would result in the risk reduction of flocks getting exposed to SE and c) reduction of SE contaminated eggs getting into the commerce.

We implemented a voluntary SE Risk Reduction Program in New England in 1989 with all producers in Maine participating. The objective of the program was to identify SE contaminated premises; to clean up these premises and to re-stock these premises with birds coming from SE test negative premises (SE clean birds into SE clean houses). All premises raising parent and pullet flocks as well as layer flocks at the end of their production cycle were tested and contaminated premises were sanitized. While single- age freestanding poultry facilities can be effectively sanitized, multiple house complexes pose a continuous challenge

The basic essentials of our SE risk reduction program are part of most programs, namely: a) NPIP SE clean breeders, b) SE clean pullet houses, c) sanitation of all contaminated premises, d) rodent and other pest control, e) feed quality and d) SE vaccinations. The implementation of these program components is extremely complex and costly and none work effectively alone. To assure their effectiveness verification is needed by microbiological testing, impartial visual inspections, pest population monitoring, serology and other measures.

The multi-million dollar 13-year clean-up effort achieved the prevention of known egg-associated SE outbreaks in humans. However, it did not succeed in a complete and sustained decontamination of all premises. Reasons for failure are as diverse as complexity of the structures, insufficient downtime, EPA or OSHA set limits of choices for disinfectants, insufficient science based information, fluctuating financial resources and commitment of management to the program as well as and cross contamination from adjacent buildings. Due to the sensitivity of the issue frank sharing of farm-based experiences does not occur adequately.

The success of these farm-based programs depends on a sustained commitment by management and program personnel, continuous implementation of all appropriate control measures and verification of their effectiveness, evaluation of the program progress and re-adjustments as needed. It requires substantial financial resources and risks.

All intensive farm programs are only at the beginning of a continuous chain of food safety responsibilities, which end with the consumer at the table.

Evaluation of Salmonella Serogroup Distributions from Commercial Broiler Houses

J. Ruiz
Department of Avian Medicine, College of Veterinary Medicine University of Georgia, Athens, GA
Co-authors: K. Burns, J. Schleifer, W.D. Waltman and C.L. Hofacre

According to recent reports of the USDA and FSIS, the incidence of *Salmonella* contamination at the carcass level in processing plants has been reduced during the last three years. In spite of the fact that processing plants have accomplish good results in this respect, it is very important for the industry to determine which levels of *Salmonella* actually come into the plant from broiler houses. In this sense, drag swab monitoring of broiler houses constitutes an accurate method of predicting potential salmonella contamination coming into the processing plants. The main objective of this study was to determine the incidence and distribution of Salmonella serogroups in randomly selected broiler farms from North Georgia and South Carolina. An evaluation of the prevalence of salmonella incidence rate and serogroups from this large broiler area of the southeast of the US will be presented.

Identification of Risk Factors Associated with the Presence of Salmonella in Post-Chill Broilers

Robert W. Wills
College of Veterinary Medicine, Mississippi State University
Co-author: Hartford Bailey

The objective of this research project was to identify risk factors that are associated with the presence of *Salmonella* in post-chill broiler carcasses. This paper will give the results of a logistic regression analysis of 2218 flocks sampled in a manner consistent with FSIS *Salmonella* performance standards tests. The independent variables used in developing the regression model included breed, age and weight of birds, hatchery of origin, time house was empty between flocks, number of flocks since litter was changed, litter condition, ventilation type, drinker type, drag swabs of house, duration of water and feed withdrawal, order of flock-processing.

Characterization of *Salmonella* from Poultry Operations

Karen A. Liljebjelke
Poultry Diagnostic Research Center, Athens, GA
Co-authors: Marie Maier, Tongrui Liu, David White, Charles Hofacre and John Maurer

Salmonella from two commercial broiler operations were characterized using PCR, Southern blot, and pulsed-field gel-electrophoresis. Six flocks yielded 295 *Salmonella* including *S. typhimurium and S. enteriditis.* Eighty percent contained class I integrons. Aminoglycoside resistance genes were identified using PCR–ELISA. There was statistically significant association between class I integrons and resistance to: sulfamethoxazole, gentamycin, tetracycline, streptomycin. Integrons are known to be a major factor in dissemination of antibiotic resistance genes in *Salmonella* and other gram negative bacteria.

***Campylobacter* Infection of Young Turkeys**

H. John Barnes
North Carolina State University, Raleigh, NC
Co-authors: Sophia Kathariou, Jean-Pierre A. Vaillancourt, Shannon M. Kozlowicz, S. Jason Hartsell

Objective:
To determine the occurrence of *Campylobacter* infection in young turkeys related to flock performance.

Design:
During this past summer 19 commercial turkey flocks were examined during brooding for growth, mortality, and presence of enteric infectious agents including *Campylobacter* spp.

Procedure:
Flocks were visited and evaluated weekly between one and 5 weeks of age. Five randomly selected poults were necropsied and one cecum was removed from each bird, placed into a sterile container, and examined for *Campylobacter* spp. by phase-contrast microscopy and culture.

Results:
Campylobacter spp. were isolated from five of 10 flocks less than two-weeks of age; all flocks greater than two weeks of age were infected. Generally once the organism was recovered from a flock, subsequent samples were also positive. At the conclusion of the study, 16 of 18 flocks (70/87 birds) remained infected. Both flocks negative at five weeks of age had been strongly positive at four weeks of age. Presence of *Campylobacter* spp. correlated with reduced growth when compared to two control groups of turkeys and a culturally negative commercial flock. Three flocks that experienced high mortality were heavily infected.

Conclusions:
In this study all turkey flocks became infected with *Campylobacter* spp., several before two weeks of age, and an association between *Campylobacter* infection and enteric disease, depressed growth, and mortality in young turkeys was found. Whether the organism is a primary or secondary pathogen, incidental, or merely an indicator of the altered intestinal tract because of intercurrent disease is unknown.

Possible Impact of Campylobacter Colonization in Commercial Turkeys

Nancy DT Reimers
College of Veterinary Medicine, North Carolina State University, Raleigh, NC
Co-authors: Katy Smith and Sophia Kathariou

Poults from a single breed source were placed into two flocks of commercial turkeys hens. Gastrointestinal and lymphoid tissues were collected for histologic examination and ceca were submitted for Campylobacter isolation from the flocks on a weekly basis. One flock remained culture negative throughout the grow-out period. Campylobacter was repeatedly isolated from the second flock beginning in the fourth week. Both flocks had low mortality levels, but the Campylobacter negative flock maintained a higher rate of growth.

Factors Affecting the Emergence of Quinolone-Resistant *Campylobacter* in Poultry

Qijing Zhang
Ohio Agricultural Research and Development Center, Ohio State University
Co-author: Naidan Luo

We conducted laboratory studies to examine the effect of use of quinolones in chicken on the development of resistant *Campylobacter*. Treatment of chickens with enroflaxacin didn't eradicate Campylobacter, but selected bacterial populations that are highly resistant to fluoroquinolones. The genetic mechanisms responsible for the resistance were also determined. These findings highlight the need for prudent use of antibiotics on poultry farms.

A Method for Determining Virulence of *Capylobacter jejuni*

Reynolds, Don
Iowa State University, Ames, IA
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Molecular Detection and Differentiation of Muscovy Duck and Goose Parvoviruses

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National Veterinary Services Laboratories, USDA, APHIS, Ames, IA
Co-authors: Dennis A. Senne and Brundaban Panigrahy

Muscovy duck parvovirus (MDPV) was first isolated in the US in 1997 following an outbreak in commercial Muscovy ducks. MDPV is antigentically closely related to goose parvovirus (GPV), a virus not present in the US. GPV is pathogenic to goslings and ducks while MDPV only affects ducklings. A nested PCR assay was developed that differentiates MDPV from GPV. The first stage primers, directed to the VPI gene, amplify both MDPV and GPV while second stage primers amplify MDPV only. The PCR assay is a rapid, specific test to detect, amplify, and differentiate MDPV from GPV directly from tissue.

Hypoglycemia-Spiking Mortality Syndrome: an Update

James F. Davis
Georgia Poultry Laboratory
Co-authors: J. T. Doman and A. E. Castro

A summary of recent cases of H-SMS in broiler and broiler breeder chickens will be presented. Data from electron microscopy, virus isolation, PCR, and bird inoculations, in the recent cases, will be presented and compared with findings reported in H-SMS cases investigated during 1992-1997.

Diseases of Quail Observed in the Alabama State Diagnostic Laboratories: 1996-2001

Frederic J. Hoerr
Alabama Department of Agriculture and Industries
Co-authors: Susan D. Lockaby, Robert Matthews, Tami Kelly, Francene Van Sambeek and Joel Cline

Quail production is an important component of animal agriculture in the southeastern United States. Most quail are produced for release on hunting preserves, but some are dressed for poultry meat markets. This presentation will discuss the most common diseases of quail seen at the Alabama State diagnostic laboratories over a 5-year period as well as offer insights to prevention and control. The diseases include: pox, quail bronchitis, mycoplasmosis, capillariasis, coccidiosis, ulcerative enteritis, cryptosporidiosis, histomoniasis, pasteurellosis, erysipelas, botulism, and various management –related disorders, and toxicities.

Association of *Pasteurella multocida* Transmission via Artificial Insemination in Turkeys

Teresa Y. Morishita
Department of Veterinary Preventive Medicine, The Ohio State University
Co-authors: Elisabeth J. Angrick and John M. Rule

Pasteurella multocida is a gram-negative bacteria that is the causative agent of avian cholera. Despite vaccination programs, the poultry industry still faces periodic outbreaks of *Pasteurella Multocida*. Although the main route of transmission is via the respiratory tract, our study indicates that transmission via the reproductive tract should not be overlooked. Outbreaks of avian cholera in turkey hen flocks have been associated with the artificial insemination of semen from infected tom turkey flocks. Moreover, the survival of *Pasteurella multocida* in turkey semen will also be presented.

Epidemiology of Fowl Cholera in Georgia

Guillermo Zavala
Georgia Poultry Laboratories Network
Co-author: Louise Dufour-Zavala

Pasteurella multocida (PM) isolates were obtained from clinical cases occurring in broiler breeders, broilers, pet birds and farm cats during 2000, 2001 and 2002. The isolates were characterized by serotyping, fingerprinting and determination of antibacterial susceptibility patterns. The seasonal and geographical occurrence of PM serotypes in Georgia is described. The pathogenicity of selected PM isolates was examined experimentally in naïve and vaccinated chickens.

Preliminary Evaluation of a Killed Polvalent Fowl Cholera Vaccine in Kestrels

Patricia S. Wakenell
University of California-Davis
Co-authors: William Ferrier and Brian A. Evans

Pasteurella multocida can be a devastating respiratory and systemic infection in both captive and wild raptors. We will present data from a preliminary study evaluating the efficacy of a killed polyvalent Pasteurella vaccine in kestrels. The kestrels were obtained from a captive population maintained at the University of California-Davis. The kestrels were challenged with a virulent field isolate of Pasteurella and evaluation of efficacy was based on clinical signs, serology, bacterial isolation and postmortem examination with histology. The challenge organism is a strain that originated in pigeons and was transmitted to various raptor species when the pigeons were used for bait in a capture/census program.

Histomoniasis in Turkeys – a Reemerging Disease?

H. L. Shivaprasad
California Animal Health and Food Safety Laboratory System, Fresno, University of California, Davis
Co-authors: R. Crespo, R. P. Chin, G. Senties-Cue, B. Charlton and G. Cooper

Objective:
To describe the incidence of Histomoniasis (Blackhead) in commercial turkeys.

Design and animals:
Data on Histomoniasis in turkeys submitted between 1989 and 2001 to the two laboratories were reviewed. Data were analyzed for the incidence, clinical signs, mortality and pathology.

Results:
No cases of Histomoniasis in turkeys were diagnosed in years 1990, 1994, 1998; 1 case was diagnosed in years 1989, 1991, 1995 respectively, 2 in 1993, 1996, 1997, and 2000 respectively, 4 were diagnosed in 1992 and 15 in 2001. Mortality was generally not significant except in 2001. In several outbreaks during 2001, the five-week mortalities ranged from 23 % to 68 %. The mortality due to Histomoniasis increased around 7 or 8 weeks of age and lasted for five weeks. Clinical signs included anorexia, depression, diarrhea and loss of weight. Gross and microscopic lesions included typhlitis, hepatitis, nephritis, and bursitis of Fabricius, splenitis, pancreatitis, proventriculitis, peritonitis and pneumonia associated with protozoa of *Histomonas meleagridis*. There was no evidence of cecal worms in the birds but round worms were found in a few cases. Treatment with Nitarsone did not alleviate the problem.

Conclusions:
The incidence of Histomoniasis was high in 2001. The five-week mortality associated with Histomonads ranged from 23 to 68 %. Treatment with Nitarsone was not effective. Cecal worms were not involved in the outbreaks.

Systemic Histomoniasis in Commercial Turkeys

Gabriel Sentíes-Cué
California Animal Health and Food Safety Laboratory Systema, Fresno Branch, University of California, Davis
Co-authors: H.L. Shivaprasad and R.P. Chin

Objective:
To describe systemic histomoniasis (Blackhead) in commercial turkeys.

Design:
Field and diagnostic investigation.

Animals:
Fifteen live and 9 dead 11 to 12-week-old meat-type male turkeys from a commercial ranch.

Procedure:
The clinical history was analyzed, birds were necropsied, gross and microscopic lesions were recorded. Microscopic examination of liver and ceca impression smears and electron microscopy of liver sections were performed.

Results:
Two out of 12 houses, each housing about 5500 turkeys, were affected. Clinical signs included anorexia, depression, diarrhea and increased mortality in turkeys between 9 and 13 weeks of age. Mortality ranged from 23 % in one house to 68 % in the other. Necropsy revealed enlargement of the livers, most of which had numerous white nodules ranging in size from 0.3 to 1.5 cm in diameter. Cecal walls were severely thickened, the lumens were distended with caseous cores and the mucosa was ulcerated. The kidneys, pancreas and spleens in a few birds had yellow foci. Microscopically, there were multifocal necrosis and granulomatous inflammation in the liver, ceca, peritoneum, bursa of Fabricius, kidneys, pancreas, proventriculus and lungs associated with Histomonads. Cytology and electron microscopy confirmed Histomonads.

Conclusions:
Histomoniasis can become systemic affecting organs other than liver and ceca. This is the first report of Histomonas affecting the bursa of Fabricius, pancreas, and proventriculus.

The DOA - A Study in Maximum Inefficiency

Kenneth N. Opengart
ConAgra Poultry Company

We reach our highest level of live production inefficiency when a chicken reaches the processing plant dead on arrival, a DOA. All production costs associated with growing the bird (feed, labor, fuel) and catch and haul have been invested and

there is little to no return. With this in mind, DOAs still may end up as an area that receives little attention because it falls at the junction of live production and plant responsibilities. This paper describes approaches used and investigations into DOA problems within several integrated complexes. It will also cover training materials designed to help plant and live production employees evaluate DOAs in a standardized format.

Assessment of a Turkey Carcass Trimming Training Program

Algis Martinez
North Carolina State University, Raleigh, NC
Co-authors: Jean-Pierre Vaillancourt and Eric Gonder

A bilingual multimedia (English/Spanish) training program on turkey carcass trimming was developed. It features texts, video clips, audio, USDA rules, references, and a quiz. The short-term impact of this program was assessed during the summer of 2001. Over trimming was determined at the slaughter plant by conducting a controlled evaluation for 6 separate groups of trimmers (evaluations performed "blind"). Each trimmer was assessed before and after training. A comparison was also performed between trained and untrained trimmers (matched by flock being processed). Each person was evaluated for three consecutive days. Results show a very significant reduction in over-trimming for all three days.

Interpretation of ELISA Titers for Georgia Broilers, Broiler Breeders and Commercial Layers

Louise Dufour-Zavala
Georgia Poultry Laboratory Network

An extensive database of ELISA titers for different antigens (NDV, IBV, REO, IBD, AE) was analyzed for Georgia commercial poultry flocks tested over two years. The results of the analysis will be discussed and interpreted in light of vaccination programs used and field problems observed during the same period of time.

Tuesday, July 16, 2002
Session A

Marek's Disease Virus in Turkeys: Experimental Infection and a Case Report From France

Andrea M. Miles
North Carolina State University College of Veterinary Medicine, Raleigh, NC
Co-authors: H. John Barnes, Jeremy S. Pittman and Jean-Luc Guerin

Since 1995, there have been reports of pathogenic Marek's disease virus (MDV) in turkey flocks in Europe and Israel. Experimental infection of turkeys with MDV and a case report of natural infection of a turkey flock in France are described.

An investigation was conducted to determine if MDV could pass horizontally between turkeys. All birds were vaccinated with HVT vaccine. Turkeys and chickens (10 per group) were inoculated at five-days of age with MDV (GA or 584A isolate) or left as controls. At three weeks post-challenge, 10 birds (same species) were added to each treatment group. Surviving birds were necropsied at nine (chickens) or 20 weeks (turkeys). MDV viremia was detected in 95%, 35%, 35%, and 0% of chicken inoculates, chicken contacts, turkey inoculates, and turkey contacts respectively. Tumors were detected in 70%, 10%, 65%, and 0% of chicken inoculates, chicken contacts, turkey inoculates, and turkey contacts respectively. These findings indicate turkeys are susceptible to MDV; they can develop viremia and virus-induced tumors. There was no evidence of horizontal transmission between turkeys.

In France, 400 chickens and 2,500 black turkeys were brooded together. The chickens were not vaccinated against MDV and experienced 90% mortality. The turkey flock had 10% mortality by 29 weeks of age. Twelve emaciated turkeys were necropsied and all had gross tumors. Histological examination showed pleomorphic populations of lymphocytes, typical of MDV induced tumors. Myelocytic cells, not typical of MDV infection in chickens, but similar to those seen in experimentally infected turkeys, were also present.

Persistence of Marek's Disease Virus in Feather Tips and Spleens of Chickens as Detected by PCR

J. Lloyd Spencer
Animal Diseases Research Institute
Co-author: Maria Chan

Commercial White Leghorn chickens were raised in isolation for three weeks and were then exposed to seeder chicks infected with the RB-1B strain of Marek's disease virus. PCR was used to detect the virus in feather tips and spleens. In 3 trials, 79 to 100% of the non-vaccinated chickens were positive for virus in feather tips after 4 weeks of contact exposure, whereas by 8 weeks, less than 50% were positive. In one trial, vaccination with turkey herpes virus reduced the number of chickens that tested positive for virus in both feather tips and spleens.

Examination of Pathogenicity and Vertical Transmission of Herpesviruses in Japanese Quail

Donald B. Link
School of Veterinary Medicine, University of California, Davis
Co-authors: Patricia S. Wakenell and Karel A. Schat

Shih et al (J. Nutr. 119:294-8, 1989) reported that embryos from quail selected for high levels of atherosclerosis were positive for MDV sequences. In two experiments the pathogenicity and possibility of vertical transmission of Marek's disease virus (MDV) was examined in Japanese quail (*Coturnix coturnix japonica*). 3-day or 5-day old quail were infected with the RB-1B strain of MDV or an MDV-like herpesvirus isolated from QT35 cells. The eggs from infected and control birds were incubated for 8, 9, 12, and 13 days, respectively, viable embryos were harvested, and DNA extracted. PCR was performed to examine the embryos for the presence or absence of MDV DNA. PCR was also performed on the splenic DNA from the uninfected and control quail. None of the infected quail developed gross MD lesions. Histopathology results will also be presented.

Mutagenesis and In Vivo Studies of Marek's Disease Virus Encoded Interleukin-8 like Gene (vIL8)

Xiaoping Cui,
Pathobiological and Diagnostic investigation, Michigan State University, East Lansing, MI
Co-authors: Lucy F.Lee, Isabel Gimeno, Willie M. Reed and Sanjay M. Reddy

Marek's disease virus (MDV) encodes a chemokine like gene designated as vIL8. This gene is located within the repeat sequences flanking the unique long region of the MDV genome. Using *recA* assisted restriction endonuclease cleavage method we have deleted both copies of vIL8 gene from MDV cosmid clones. We have also constructed mutants in which the "DKR" (Asp-Lys-Arg) motif in vIL8 was changed to a chemokine conserved "ELR" (Glu-Leu-Arg) motif. It is known that chemokines with an ELR motif are able to attract the migration of neutrophils (heterophils in chicken). Transfection of these mutated cosmids into cells in culture resulted in the generation of recombinant MDVs. The first recombinant has both copies of vIL8 deleted and the second has the DKR motif changed to ELR. The construction and biological characteristic of these recombinants will be discussed.

Role of 1.8 kb mRNA Family of Transcripts in Marek's Disease Virus Pathogenesis

Robert F. Silva
Avian Disease and Oncology Laboratory, USDA, ARS
Co-authors: Blanca Lupiani and Sanjay M. Reddy

Oncogenic Marek's disease virus (MDV) can be attenuated by repeatedly passing the virus in cell culture. Attenuation is also associated with the increase in the number of copies of a 132 bp repeated region located in TRL and IRL of the viral genome. We have mutated this region of the MDV genome and will demonstrate how these mutations affect viral virulence.

History of Biological Control of Poultry Diseases in the U.S.

Stephen B. Hitchner

Much of the success and growth of the poultry industry can be attributed to the cooperative effort of research institutions, the laboratories that provided the biologics and the federal government supervision to assure the safety and effectiveness of the

products. The discussion will be confined to those biologics which have been approved by the USDA, beginning with the pullorum/typhoid testing program. Subsequent discussions will include the viral vaccines, methods of production and the advancements made in the methods of application to meet the needs of the industry.

Immunologic Tolerance in Chickens Hatching from Eggs Injected with Cell-associated Turkey Herpesvirus (HVT)

Jagdev M. Sharma
College of Veterinary Medicine, University of Minnesota, St. Paul, MN
Co-author: Yugen Zhang

We inoculated cell-associated HVT in eggs at various stages of incubation. Chickens hatching from these eggs were tested for anti-HVT antibodies by several serologic methods and those remaining free of detectable antibodies were considered tolerant to HVT. Chickens exposed to HVT at embryonation day 14 or earlier had 6-33% incidence of tolerance. Tolerant chickens developed persistent HVT viremia and showed reduced resistance to virulent Marek's disease virus. Tolerance to HVT did not influence the ability of the chickens to produce antibodies against an extraneous antigen or respond to a T cell mitogen.

Immunological Features of Serotype 1 Marek's Disease Virus Vaccines

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Co-authors: Witter, R.L. Hunt, H.D. and Reed, W.M.

In an attempt to evaluate immunological parameters that might be related with level of protection against Marek's disease, the immune responses induced by eight serotype 1 Marek's disease virus vaccines conferring different levels of protection have been characterized. A chronological study of several immunological parameters including natural killer activity, frequency of different lymphocyte subsets and expression of major histocompatibility complex (Mhc) class I and class II antigens was conducted. Compared to other vaccines, vaccines conferring the highest level of protection seemed to induce an earlier activation of the immune system (i.e. activation of T cells measured by the level of Mhc-II antigen expression, expansion of T cells CD4+ and greater natural killer cell activity). Pathogenic strains of serotype 1 MDV also induced early activation of the immune system. However, in contrast to vaccine strains, pathogenic viruses significantly reduced the level of both B cells and Mhc-II+ T cells by 7 days post inoculation due to a strong cytolytic infection of these cell types. Application of these findings in evaluating vaccine strains will be discussed.

Influence of *B*-haplotype on the Efficacy of Recombinant Fowlpox Vaccine Protection Against Marek's Disease in Chickens

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Co-authors: Larry D. Bacon, Sanjay M. Reddy, and Richard L. Witter

Recombinant fowlpox virus (rFPV) containing glycoprotein gB genes from three serotypes of Marek's disease virus (MDV) was used to study the influence of *B*-haplotype on vaccine responses in chickens. Sequence analysis of the gB gene from three serotypes showed 80% homology. Chickens were vaccinated intraperitoneally at hatch. At 6 days of age, the chicks were challenge with 500 pfu of Md5. The level of protection by gB of three serotypes was significantly different in *B* congenic lines of chickens. The level of protection in chickens was highest for $B_{21}B_{21}$, intermediate for $B_{13}B_{13}$ and lowest for B_5B_5. In two additional trials, a rFPV containing four MDV genes was used to study the efficacy of protection in chickens of different *B* haplotypes. The result confirms that $B_{21}B_{21}$ chickens provide the best protection against challenge with several MDV viruses. These results taken together indicate a significant influence of *B* haplotypes on vaccine responses resulting from rFPV.

Development of New Attenuated Serotype 1 Vaccines with Improved Efficacy Against Marek's Disease through Cell Culture Passage, Chicken Backpassage, and Retroviral Insertion

Richard L. Witter
Avian Disease and Oncology Laboratory, USDA, ARS, East Lansing, MI

A battery of 10 candidate serotype 1 vaccines against Marek's disease (MD) was developed using classical virological methods. Each vaccine was evaluated for protective efficacy and safety. Virus strains included both v and vv+ pathotypes. Candidate vaccines were derived by several strategies, including attenuation by cell culture passage, use of partially attenuated strains, manipulation of virulence by backpassage in chickens, and use of natural mutants containing retroviral LTRs. Protection was compared to that of licensed vaccines, especially strain CVI988/Rispens. Several vaccines provided protection equal to or greater than that of CVI988/Rispens. Whereas some of these vaccines induced lymphoid organ atrophy or lymphoproliferative lesions in susceptible chicks lacking maternal antibodies, even the most aggressive of these vaccines induced little or no pathology in commercial chicks with maternal antibodies. These studies illustrate that development of new vaccines with yet higher levels of protective immunity against MD virus challenge is feasible. These strategies may help in the realization of the next incremental improvement in MD vaccines.

Localization and Profiling of Reticuloendotheliosis Virus (REV) Protein/s in Cultures Infected with REV Integrated Fowlpox Virus (Rev-FPV) Strains

Theodros Tadese
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Co-author: Willie Reed

Commercial flocks previously vaccinated are susceptible and have continually been infected with variants or field isolates of fowlpox virus (FPV). These field isolates and a number of vaccine strains are now known to have been intimately associated and integrated with reticuloendotheliosis virus (REV) in the genome.

To understand the nature of association between REV and FPV strains, a study is currently underway in our laboratory to isolate and characterize specific REV antigen/s in cultures infected with FPV strains. One dimensional (1-D) gel and immunoblotting (IB) and immunofluorescence (IF) are being used to profile and localize these antigen/s from field isolates and vaccine strains. These techniques are also being used to compare and further characterize and evaluate antigen/s of interest targeted as a potential immunodominant proteins for the development of an efficacious vaccine against FPV.

Molecular and Biological Characterization of Naturally Occurring Recombinant Avian Leukosis Viruses (ALV) Isolated from Egg-type Chickens Suffering from Myeloid Leukosis

Blanca Lupiani
Animal Health and Diagnostic Laboratory, Michigan State University, East Lansing, MI
Co-authors: Arun R. Pandiri, Henry Hunt, Willie M. Reed and Aly M. Fadly

A case of myeloid leukosis has recently been reported in commercial layers. Molecular characterization of viruses isolated from affected chickens proved that they are recombinant viruses with subgroup B avian leukosis virus (ALV) envelope and subgroup J ALV LTR. The recombinant virus termed ALV-J/Benv replicated in chicken embryo fibroblasts (CEF) that are known to be resistant to ALV-J, but failed to replicate in CEF known to be resistant to subgroup B ALV. Chickens of line 15 X 7 known to be highly susceptible to ALV infection and tumors were inoculated with ALV-J/Benv at hatch; chickens were monitored for ALV infection and tumors for 30 weeks. The tumor incidence in ALV-J/Benv-inoculated chickens was 40% compared with 20% and 76% in chickens inoculated with strain ADOL-Hc1 of ALV-J or RAV-2, a subgroup B ALV, respectively. Results suggest that natural recombination among various subgroups of ALV may influence their ability to induce tumors.

Quantitation of Viral RNA and Proviral DNA Levels of Subgroup J Avian Leukosis Virus (ALV-J) by Real-Time RT-PCR and PCR

Arun R. Pandiri
Department. of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI
Co-authors: Blanca Lupiani, Willie M. Reed and Aly M. Fadly

An assay for the quantitation of viral RNA and proviral DNA of subgroup J avian leukosis virus (ALV-J) was developed. Primers were designed on gp85 region of the viral envelope and detected all of the ALV-J isolates tested in this study. This assay can be used to quantitate viral RNA directly from plasma and tissue culture supernatants without the need to separate the nucleic acid, however, quantitation of proviral DNA requires prior isolation of the nucleic acid. Results suggest that direct real-time quantitation of viral RNA is a quick and more accurate method that can be used for diagnosing and quantitating ALV-J viremia levels than traditional tissue culture based methods.

The Design and Evaluation of Antisense Oligomers as Potential Inhibitors of Avian Leukosis Virus Replication

John El-Attrache
The University of Georgia, Athens, GA
Co-authors: Pedro Villegas, Scott Callison and Alejandro Banda

Antisense phosphorodiamidate morpholino oligomers (MOs) have been analyzed as potential inhibitors of avian leukosis virus subgroup J (ALV-J) replication. Inhibition of replication was analyzed in vitro by the p27 antigen capture-enzyme linked immunosorbent assay (ec-ELISA) from cell-free supernatant of DF-1 cells infected with ALV-J pre- and post-treatment with the appropriate morpholinos. A significant reduction ($p<0.05$) in the production of p27 was observed for the MO targeted to the ALV primer binding site when compared to the treatments with a non-specific MO control. A one log reduction in viral RNAs was observed by Real time LightCycler RT-PCR and correlated with a one log reduction of infectious units observed when an endpoint dilution assay was performed for the same treatment.

Studies of Subgroup J Avian Leukosis Virus Infection and Tumors in a Naturally Infected Commercial Broiler Breeder Flock

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Co-authors: Arun R. Pandiri, Larry D. Bacon and Aly M. Fadly

Chickens were pedigree hatched from a commercial broiler breeder flock that had been identified by the company to have a relatively high incidence (20% - 60%) of subgroup J avian leukosis virus (ALV-J) infection. Unexpectedly, only one of 175 (0.6%) of chicks hatched at our laboratory tested positive for ALV-J at hatch; however, 55/97 (56%) of these chicks tested positive for endogenous ALV. At various ages, the incidence of viremia and cloacal shedding varied from 8% - 16% and from 1% - 13%, respectively. Only two of 157 (1.3%) of chickens developed tumors by 32 weeks of age. Results suggest that the virus had spread to 16% of the hatchmates, with only 5% persistently viremic. The relatively high incidence of ALV-J infection initially reported by the breeder company was likely due to the presence of endogenous ALV.

Response of Chickens from Three Commercial Broiler Breeders and Two Experimental Lines to Infection with A Field Strain of Subgroup J Avian Leukosis Virus

Aly M. Fadly
USDA-Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI
Co-authors: Jody K. Dybing and Arun R. Pandiri

Chickens from three commercial broiler breeders and two experimental lines were inoculated as embryos with strain ADOL-6803 of subgroup J avian leukosis virus (ALV-J). At various ages, chickens were tested for ALV-J-induced viremia, antibody and cloacal shedding; chickens were also monitored for ALV-J-induced tumors through 28 weeks of age. The incidence of viremic-tolerant chickens varied from 67% - 100%, whereas the incidence of tumors varied from 46% - 84%. Results suggest that chickens from commercial broiler breeders, regardless of breed were more susceptible to ALV-J infection and tumors than chickens from two experimental lines.

Effects of Injecting ALV-J Antiserum in Embryonating Broiler Eggs on Localization of ALV-J in the Tissues of Broiler Chickens Infected *in ovo*

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Co-author: Tom P. Brown

The protective effects of injecting antiserum against subgroup J avian leukosis virus (ALV-J) into embryonating-chicken eggs before infection were determined by evaluating viremia, transfer of passive immunity, and localization of the virus in tissues from hatched chicks. None of the chicks hatched from eggs inoculated with ALV-J antiserum had detectable antibodies by virus neutralization test; however, the injected antiserum prevented viremia at hatch in four out of five chicks. Localization of ALV-J in chicks that were viremic at hatch was similar to previous investigations with intense staining for viral antigen present in adrenal gland, heart, kidney, proventriculus, and spleen. Two of the chicks that were not viremic at hatch developed viremia at 1 week of age and had viral tissue distribution suggesting oral exposure to the virus from their hatch mates.

Incidence of Cancer in Layer Hens and Effects on Egg Production

Gina F. Alban-Martinez
Poultry Science Department, North Carolina State University, Raleigh, NC
Co-authors: Donna K. Carver and H. John Barnes

Geriatric laying hens were monitored for incidence of cancer. A total of 2277 hens have been monitored since 52 weeks of age. As mortality occurs in the flock, gross necropsy and histopathology are used to assess the presence and type of cancer in each bird. An overview of the types and incidences of cancers present in geriatric birds will be presented. Additionally, the effects of each cancer type on egg production will be discussed.

Mortality Patterns and Etiologies in Geriatric Laying Hens

Donna K. Carver
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Co-authors: Gina F. Alban-Martinez and H. John Barnes

Twenty four hundred laying hens were monitored for one-year period to determine mortality patterns and etiologies. The hens were entered into the study at 2 years of age and followed until the flock was three years of age. Patterns of mortality will be described related to age and changes in management. Etiologies will be summarized and both infectious and non-infectious causes of disease will be discussed. These findings could have implications in decision-making regarding re-cycling layer hens.

Tuesday, July 16, 2002
Session B

Avian Pneumovirus (APV) RNA Isolated from Seagulls Shows Genetic Homology with RNA from APV Isolates from Domestic Turkeys

Binu Belayudham
Co-authors: Vanessa Lopes, David Halvorson and Kakambi Nagaraja

Avian pneumovirusis the causative agent of an acute respiratory illness that mainly affects turkeys. Respiratory disease caused by avian pneumovirus first appeared in USA in 1996 and it is an economic concern for the people associated with turkey industry. The disease is characterized by coughing, sneezing, nasal discharge, tracheal rales foamy conjunctivitis and swollen sinus. APV viral RNA was detected in the nasal turbinates of seagulls collected from the vicinity of a turkey farm. The virus was detected by RT-PCR in the nasal turbinate homogenate. The M gene isolated using primers 5'-ATGGAGTCCTATCTAGTAG-3' and 5'-CTAAATAATATCAAGCTAGG-3' was then cloned in pGEMT EASY vector and sequenced. The results indicated a high nucleotide sequence similarity and predicted amino acid sequence identity with that of APV isolated from domestic turkeys. The finding suggests the possible involvement of seagulls in the transmission of the virus in domestic turkeys.

Effect of Age of Turkeys on Susceptibility to Avian Pneumovirus-induced Acute Disease and Mitogenic Inhibition

Heather Gerbyshak-Szudy
Co-authors: Hayet Abbassi and Jagdev Sharma

Oculonasal exposure of turkeys to virulent avian pneumovirus (APV) causes mild to moderate respiratory signs and immunosuppression during one through five days post-infection. We exposed turkeys to virulent APV at two, four, six, eight, and ten weeks of age. More than half of the exposed turkeys showed clinical signs after exposure at two to six weeks of age. One-third showed clinical signs at eight weeks, and none at ten weeks. Severity of clinical signs decreased steadily with age. In addition, mitogenic inhibition of spleen cells was detected in birds exposed to APV at two and four weeks of age but not in those exposed at six or ten weeks of age. We conclude that less severe disease is not associated with mitogenic suppression. Although APV infection may occur in turkeys of all ages, acute disease and immunosuppression is restricted to younger turkeys.

Development and Evaluation of a Competitive ELISA for the Detection of Avian Pneumovirus Antibodies in Various Avian Species

Elizabeth A Turpin
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Co-author: David E. Swayne

Avian pneumovirus (APV) were first isolated in the United States in 1997. The virus has continued to result in respiratory disease and subsequent economic loss since its emergence. The sudden appearance of APV in the U.S. has led to the speculation that wild birds may play a role in transmission. To determine the presence and extent of APV infection in wild bird populations our lab has developed a competitive ELISA. Sera samples from wild birds from throughout the U.S. are being screened. The competitive ELISA has been validated using experimental and field samples. This test is advantageous in its ability to rapidly detect APV antibodies from numerous samples, independent of bird species.

Role of Local Cellular Immunity in Protection Against Respiratory Challenge with Avian Pneumovirus

Parag Chary
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Co-authors: Mathur S Kannan and Jagdev M Sharma

Turkeys exposed to APV by occulonasal route showed clinical signs and lymphoid cell infiltration in the upper respiratory tract (URT), especially histopathology characterized by a marked increase of cells in the Harderian gland (HG). The virus genome was detectable by PCR in the choanal swabs. Lymphoid cells recovered from URT had increased interferon gamma (IFN-γ), increased levels of NOIF and decreased calcium uptake in comparison with virus free control. Turkeys recovered from occulonasal exposure were resistant to subsequent occulonasal challenge with the virulent virus. Subcutaneous exposure to APV did not cause clinical signs or detectable cellular response in the URT. Turkeys given APV subcutaneously were not protected against a challenge with virulent APV given occulonasally.

Avian Pneumovirus Disease (APV) Outbreaks in Turkeys in the Midwest: A Field Perspective

Hugo A. Medina
Jennie-O Turkey Store

APV in turkeys has been identified since 1996 in the Midwest (Minnesota, Iowa, South and North Dakota). Advances in virus isolation, PCR, serology (ELISA), histopathology and virus passages have provided with tools to better understand, control and minimized the disease effects in turkeys.Most of the information has been concentrated on the isolation, identification, virulence of the virus isolates, characterization, serology response, possible pathways of transmission, and severity of the disease. Emphasis on biosecurity practices from the breeder to the processing plant. All these have help better understand some of the possible ways how the disease is introduce and some of its effects on infected birds. This field perspective is primarily concentrated on the potential influence of other factors in the presence of APV in a flock, farm and/or area. We will present the influence and/or effects of APV from biosecurity, other disease problems, seasonal influences, vaccinations, environmental factors, therapeutic treatments, densities, APV killed day old vaccination in poults, breeder vaccination and other preventive measures. Review of serological profile on APV vaccination in breeders and on day old and non-vaccinated poults.

Evaluation of a Wild Bird Avian Pneumovirus Isolate as a Vaccine Candidate

David A. Halvorson
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Co-authors: Richard S. Bennett, Moses K. Njenga and Kakambi V. Nagaraja

Avian pneumovirus was isolated from wild Canada geese. One of the isolates was administered to turkeys and the turkeys were then challenged with a turkey isolate of APV. This presentation will describe the effects of both the wild bird isolate and the challenge virus on the turkeys.

A Comparison of Diagnostic Tests Performed on Brooding Age Poults and Designed to Identify Pems Agents

S. Kozlowicz
College of Veterinary Medicine, NC State University, Raleigh, NC
Co-authors: J. Hartsell, D. Rives, M. Garcia, J. Guy, J. Barnes, S. Schultz-Cherry and J.P. Vaillancourt

Poult Enteritis Mortality Syndrome (PEMS) is a severe enteric disease that has impacted the North Carolina turkey industry since 1990. To date, the exact etiology is unknown; however, several viruses are being investigated as potential causal agents. The purpose of this study was to determine the association between PEMS and three viruses shown to produce PEMS under laboratory conditions; coronavirus, astrovirus, and reovirus.

In 2001, a study was conducted on 43 turkey flocks located in North Carolina, Virginia, and Pennsylvania. For each flock, sample collection consisted of five live birds for necropsy, 20 blood samples, and 50 body weights. Samples were first collected when birds were one week old and continued weekly for five weeks. Daily mortality was also recorded. Tissues and sera were sent to laboratories that have developed diagnostic tests for potential PEMS agents. Coronavirus status was determined by serology using ELISA. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used on fecal samples for astrovirus and reovirus.

Eleven flocks were diagnosed with PEMS based on the mortality data. All flocks included in this study tested negative for coronavirus. Mortality was about 7% in astrovirus positive flocks as compared to about 4% in negative flocks. These results were significant ($p<0.1$). Reovirus status, on the contrary, was not significantly associated with mortality ($p=1.0$). Reovirus positive flocks showed about 4.5% mortality over 5 weeks, while the negative flocks showed about 5.5% mortality. The astrovirus status of each flock was determined using RT-PCR on direct samples and after passage through embryos. A comparison of these two tests showed that the PCR after embryo passage was more sensitive than with direct samples (Se=95%), but it was also much less specific (Sp=27%). Therefore, the PCR on direct samples is recommended. The results of this study support laboratory findings that astrovirus is one of the causative agents but indicate that reovirus and coronavirus are not necessary factors for producing PEMS in commercial flocks. This is especially important with regards to coronavirus, which has long been implicated as a causative agent of PEMS. Although a coronavirus infection in the laboratory may produce clinical signs of PEMS, field data from 1996, along with this current data, shows this is not the case. These results are an important step in elucidating the agents responsible for causing PEMS in commercial poultry operations. In addition, this study has compared the results of different laboratory procedures for the same virus so that we may utilize a better diagnostic tool in the future. We must elucidate the responsible agents and have sensitive and specific diagnostic tests to provide effective control and preventative measures to overcome the problems of decreased weight and increased mortality associated with Poult Enteritis Mortality Syndrome.

Brooding Performances in 43 Turkey Flocks Depending on Diagnostic Results from Test Developed for PEMS Related Agents

Jean-Pierre Vaillancourt
North Carolina State University, Raleigh, NC
Co-authors: John Barnes, Stacey Schultz-Cherry, Mo Saif, Tom Hooper, Jim Guy, Shannon Kozlowicz, Jason Hartsell, Nancy Reimers, Marion Garcia and David Rives

A study was conducted in 2001 on 43 turkey flocks located in North Carolina, Virginia, and Pennsylvania. For each flock, a weekly collection consisted of: five live birds for necropsy, 20 blood samples; 50 body weights. . Daily mortality was also recorded. Tissues and sera were sent to laboratories that have developed diagnostic tests for PEMS related agents. This protocol allowed us to determine performances relative to diagnostic test results under field conditions. Of the different agents

considered (coronavirus, astrovirus, reovirus, rotaviruses, Salmonella, cryptosporidia, etc.), Astrovirus was the most strongly associated with mortality. Results for all agents will be presented.

Use of a Human Cell Line to Induce Helper Virus-Free Replication of an Avian Adeno-Associated Virus

Carlos Estevez
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Co-authors: Pedro Villegas and John El-Attrache

DNA vaccines and gene therapy have become mainstream in recent years due, in part, to the great advances achieved in the area of genomics and molecular engineering. One of the main problems with these procedures is the delivery of the DNA material to the target tissue. As a first step in the development of a safe viral vector for delivery of such DNA material in chickens, we have attempted to replicate the avian adeno associated virus strain AR-865 in a human kidney cell line that expresses the immediate early genes of a human adenovirus type 5. Experimental work is currently ongoing.

Stimulation of the Innate Immune System of Broilers with DNA Oligonnucleotides Containing CpG Motifs (CpG DNA)

Susantha M. Gomis
Veterinary Infectious Disease Organization
Co-authors: Dale Godson, Lorne Babuik, Brenda Allan, Rolf Hecker and Andrew Potter

Short DNA oligonucleotides containing CpG motfis (CpG-DNA) have been shown to be effective immunoprotective agents in murine models. The objective of this study was to investigate the effect of CpG-DNA against cellulitis / colibacillosis in a broiler model. The control group of birds that received no CpG-DNA had a survival rate of 15%. In contrast, groups that received CpG-DNA by subcutaneous or intramuscular injection had significantly higher survival rates. The size of the cellulitis lesion was significantly smaller in groups that received CpG-DNA by subcutaneous route ($p<0.01$). This study demonstrated that CpG-DNA has both localized and systemic immunoprotective effects in broilers.

Dactylariasis in Broiler Chickens

Francene S. Van Sambeek
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Co-authors: Susan D. Lockaby and Burton Maxfield

Twelve days of age, broiler chickens from a north Alabama multiplier company were submitted for central nervous system signs, trembling, apparent blindness and head shaking. Postmortem lesions included pericarditis, perihepatitis, airsacculitis, brain discoloration and excessive brain fluid.

Collection of the brain swabs was performed in both the field and under a biosecurity level 2 hood. Cultures on both sheep blood agar and Sabourand dextrose agar revealed a wooly, reddish-brown colony with a purple reverse. Microscopically, the septate fungal growth was consistent with *Dactylaria contricta* variant *gallopava*.

Histopathology findings were a necrotizing granulomatous encephalitis with fungal hyphae consistent with dactylariasis.

***Mycoplasma gallisepticum* in Commercial Layers in Pennsylvania**

Sherrill Davison
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Co-authors: Stanley S. Kleven, Eric N. Gingerich and Robert J. Eckroade

The Pennsylvania layer industry continues to have chronic losses in table egg production due to *Mycoplasma gallisepticum* (MG) despite vaccination.

To determine if a flock is truly positive for MG, tracheal samples are taken for the polymerase chain reaction assay, culture and typing. The PCR test may produce negative results even though the flock is suspected of having MG. In addition, obtaining a pure culture of MG from layers is extremely difficult and expensive because the majority of cultures are

overgrown by non-pathogenic mycoplasmas. To eliminate this problem in cultures, Dr. Stanley Kleven suggested the novel approach of the use of sentinel turkeys as "filters" for the MG. Layers suspected of being infected with MG are placed with mycoplasma-free turkeys. The turkeys are then cultured for MG rather than culturing the chickens.

The objectives of this study is to survey selected layer flocks in multiple-age complexes for *Mycoplasma gallisepticum* using sentinel turkeys and to type the *Mycoplasma gallisepticum* isolates cultured from the sentinel turkeys and determine their pathogenicity.

Presence of Pathogenic Mycoplasmas in Free-Flying Visitor Birds in Commercial Layer Farms from the South Peruvian Coastal Area (Chincha)

Norma Noé
College of Veterinary Medicine, University of San Marcos
Co-authors: Eliana Icochea, Mónica Alba, Rosa González, Mirtha Roque and Sharon Levisohn

Previous studies conducted in 300 commercial layers from 14 flocks located in Chincha Province, showed a prevalence of 94% and 58% for Mycoplasma synoviae and Mycoplasma gallisepticum infection, respectively. The objective of the study was to evaluate the Mycoplasma status of free-flying migratory visitor birds from commercial layer farms in Chincha. Eyes, tracheal and choanal cleft swabs, were taken from free-flying visitor birds to detect Mycoplasma using PCR. Blood samples were collected for the detection of antibodies to MG and MS by plate agglutination test.

Diagnosis of Mycoplasma Infection in Colorado (1999 to present)

Brian Wooming
Longmont Foods Live Production Lab
Co-authors: David Ley and Sile Huyan

The presentation will compare on serologic, bacteriologic and molecular methods used in the diagnosis of Mycoplasma infections in Colorado turkeys. The predictive value of each test will be discussed.

A House Finch-Like *Mycoplasma gallisepticum* Outbreak in a Midwest Turkey Company

David R. Hermes

A *Mycoplasma gallisepticum* infection occurred in a hen turkey breeder flock in Indiana. Blood surveillance consisted of serum plate agglutination (SPA) screening with Hemagglutination Inhibition (HI) confirmation of reactors. Prior to the onset of clinical signs, no MG positive sera had been found.

The MG was discovered when the farm manager noticed a slight increase in mortality in one house. Although the mortality was diagnosed as fowl cholera, a very small number of birds had swollen infraorbital sinuses. No respiratory noise was present. Tracheal swabs were 100% PCR positive for MG at two different labs. SPA and HI were still negative at this time. Based on the PCR and clinical signs, the entire farm was depopulated within two weeks. Eggs that were collected after the last routine serological test (approximately three weeks prior to the detection of clinical signs) were removed from the hatchery and destroyed.

Continued serological and culture efforts were made prior to slaughter. MG culture was eventually successful at two labs from samples taken during this period. Seroconversion on SPA and HI was very weak even two weeks after the onset of clinical signs, seldom higher than 1:20 on HI. A few samples were 1:40 at one lab.

DNA fingerprinting showed a strong similarity with the House Finch MG strain. Meat-bird progeny from this breeder flock eventually showed mild clinical signs as they neared slaughter age. However, airsacculitis was not an issue at processing.

The Characterization of a Naturally Occurring Mycoplasma gallisepticum House Finch-like Strain from Commercial Turkeys

Naola M. Ferguson
Department of Avian Medicine, University of Georgia, Athens, GA
Co-authors: David Hermes, Victoria A. Leiting and Stanley H. Kleven

A *Mycoplasma gallisepticum* (MG) isolate, designated K5054, from an outbreak in commercial turkeys was isolated from a bioassay. It was found to be similar to house finch strains by random amplified polymorphic DNA (RAPD) analysis and sequence analysis of portions of the phase-variable putative adhesion protein (pvpA) gene, a lipoprotein gene (LP), and the cytadhesin gapA gene. The isolate was also evaluated for virulence, transmission and immunogenicity in turkeys.

A Preliminary Investigation of a Mycoplasma Gallisepticum House Finch-like Strain as a Vaccine in Turkeys

Naola M. Ferguson
Department of Avian Medicine, University of Georgia, Athens, GA
Co-authors: Victoria A. Leiting and Stanley H. Kleven

A *Mycoplasma gallisepticum* (MG) isolate from an outbreak in commercial turkeys was found to be similar to house finch strains by DNA fingerprinting and sequence analysis. In a preliminary study, this isolate was found to cause very mild lesions and to render protection against subsequent challenge with a virulent MG strain. In this study, this isolate, designated K5054, was further evaluated as a potential vaccine in layer chickens and turkeys.

Sequencing of an *iss*-Containing Fragment from a Conjugative R Plasmid of an Avian *Escherichia coli* Isolate

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Co-authors: Catherine W. Giddings, Shelley M. Horne and Lisa K. Nolan

Summary:
Avian colibacillosis causes multi-million dollar losses for the poultry industry annually. Yet, the etiological agent of this disease, *Escherichia coli*, has virulence mechanisms that are not well understood. However, the last decade of research in this field has brought about the discovery of some common attributes of avian *E. coli* in an effort to control avian colibacillosis. One of these attributes is the possession of the *i*ncreased *s*erum *s*urvival gene (*iss*). *iss*, first described by Binns et al for its role in the complement resistance associated with a ColV plasmid of a human *E. coli* isolate, has been shown to increase the virulence of an *E. coli* 100-fold for day-old chicks and its complement resistance greater than 20-fold. Several studies have suggested that *iss* occurs much more frequently in isolates implicated in avian colibacillosis than it does in fecal *E. coli* of healthy birds. Recent findings in our lab have localized *iss*, along with several other genes thought to be involved with avian *E. coli* virulence, to a single transmissible R plasmid in an avian isolate. In the present study, an *iss*-containing fragment of this plasmid that was obtained by digestion with *Eco*RI was subcloned, sequenced and analyzed in order to provide more insight into the virulence mechanisms of avian *E. coli*. Procedures involved in the sequencing of this plasmid and implications of the results will be discussed.

Production of Monoclonal Antibodies Against Avian *Escherichia coli* Iss

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Co-authors: Steven L. Foley and Lisa K. Nolan

We have found that the presence of *iss* DNA sequences is strongly correlated with *E. coli* implicated in avian colibacillosis, making *iss*, and the protein it encodes (Iss), candidate markers of avian *E. coli* virulence. As part of an overall effort to explore potential colibacillosis control strategies, monoclonal antibodies (Mabs) to Iss have been generated by immunizing mice with calmodulin binding peptide-Iss (CBP-Iss). Hybridomas were produced by the fusion of spleenocytes from the hyper-immune Balb/C mouse and SP2/O mouse myeloma cells. *E. coli* isolates known to possess or lack *iss* sequences have been screened using the Mabs in ELISA and Western blotting protocols in order to determine the potential utility of these Mabs in detection of virulent avian *E. coli*.

Adaptation of Virulent Serotype I Infectious Bursal Disease Virus to Avian Macrophages

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Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul, MN
Co-authors: H. Gerbyshak-Szudy, H.Y. Yeh and J.M. Sharma

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A Possible New Serotype 1 Variant of Infectious Bursal Disease Virus from Korea

Sun-Joong Kim
Co-authors: Hyuk-Joon Kwon, Sun-Hee Cho, Eun-Kyoung Lee, Min-Chul Kim and Dong-Woo Lee

An infectious bursal disease virus (IBDV) was isolated from a broiler flock in Korea. The virus induced bursal atrophy without mortality in susceptible chickens. The nucleotide sequence of the VP2 region of the virus located the virus in a separate position in the phylogenetic trees for the hypervariable region as well as the whole length, distinct from the known classical and variant IBDVs of serotype 1. The virus was also differing in the predicted restriction fragment length polymorphisms from the known IBDVs.

Identification of a Variant IBDV Strain that Behaves Clinically as a Classic using Histopathology, PCR, and Monoclonal Antibodies in Commercial Broilers in Peru

Manuel Contreras,
Merial
Co-authors: Lui Alzamora, Stephanie Mengel Whereat and Fernandez Rafael

380 histopathology slides and 55 PCR from bursal tissues were tested at the University of Georgia and more than 500 post-mortem exams were evaluated in the field in Peru for a period of 18 months after reports of the presence of an immunosuppressive agent. More than 50% of the PCR tested were identified as variants and close to 25% as classic. Using the monoclonal antibody technique developed by Snider and Mengel, currently run by the University of Delaware, more than 42% of the samples examined were identified as variants. In spite of these findings, the lesions reported in all cases occurred between 21 and 28 days and consisted of caseous material, edema and hemorrhage (classic IBD). No atrophy was reported the first 3 weeks of life.

Mild, Intermediate and Virulent Strains of Infectious Bursal Disease Virus Differ in their Ability to Modulate the Immune System

Silke Rautenschlein
Klinik für Geflügel, Tierärztliche Hochschule Hannover, Germany
Co-authors: Hung-Yueh Yeh and Jagdev M. Sharma

Infectious bursal disease viruses (IBDV) of different virulence such as virulent IBDV-IM, intermediate IBDV-B2 and IBDV-Lukert, and mild IBDV-BVM were compared in their ability to stimulate and modulate the immune system of chickens. We evaluated systemic parameters such as induction of anti-IBDV-antibodies, circulating nitrite levels and spleen cell mitogenic response as well as local intrabursal events such as T cell accumulation and cytokine induction. The level and time point of induction of circulating virus-neutralizing antibodies (VN) correlated with the virulence of the IBDV-strain. IBDV-IM induced the highest ELISA antibody levels detected at days 8 to 29 post infection (PI), while IBDV-BVM had the latest and the lowest VN antibodies levels. IBDV-IM stimulated systemic circulating nitrite levels and suppressed the splenic mitogenic response the most in comparison with other strains. Interestingly, no difference were observed between virulent and intermediate strains in the induction of local intrabursal T cell accumulation and the expression T cell related cytokines. We

The Characterization of a Naturally Occurring Mycoplasma gallisepticum House Finch-like Strain from Commercial Turkeys

Naola M. Ferguson
Department of Avian Medicine, University of Georgia, Athens, GA
Co-authors: David Hermes, Victoria A. Leiting and Stanley H. Kleven

A *Mycoplasma gallisepticum* (MG) isolate, designated K5054, from an outbreak in commercial turkeys was isolated from a bioassay. It was found to be similar to house finch strains by random amplified polymorphic DNA (RAPD) analysis and sequence analysis of portions of the phase-variable putative adhesion protein (pvpA) gene, a lipoprotein gene (LP), and the cytadhesin gapA gene. The isolate was also evaluated for virulence, transmission and immunogenicity in turkeys.

A Preliminary Investigation of a Mycoplasma Gallisepticum House Finch-like Strain as a Vaccine in Turkeys

Naola M. Ferguson
Department of Avian Medicine, University of Georgia, Athens, GA
Co-authors: Victoria A. Leiting and Stanley H. Kleven

A *Mycoplasma gallisepticum* (MG) isolate from an outbreak in commercial turkeys was found to be similar to house finch strains by DNA fingerprinting and sequence analysis. In a preliminary study, this isolate was found to cause very mild lesions and to render protection against subsequent challenge with a virulent MG strain. In this study, this isolate, designated K5054, was further evaluated as a potential vaccine in layer chickens and turkeys.

Sequencing of an *iss*-Containing Fragment from a Conjugative R Plasmid of an Avian *Escherichia coli* Isolate

Timothy J. Johnson
Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND
Co-authors: Catherine W. Giddings, Shelley M. Horne and Lisa K. Nolan

Summary:
Avian colibacillosis causes multi-million dollar losses for the poultry industry annually. Yet, the etiological agent of this disease, *Escherichia coli*, has virulence mechanisms that are not well understood. However, the last decade of research in this field has brought about the discovery of some common attributes of avian *E. coli* in an effort to control avian colibacillosis. One of these attributes is the possession of the *i*ncreased *s*erum *s*urvival gene (*iss*). *iss*, first described by Binns et al for its role in the complement resistance associated with a ColV plasmid of a human *E. coli* isolate, has been shown to increase the virulence of an *E. coli* 100-fold for day-old chicks and its complement resistance greater than 20-fold. Several studies have suggested that *iss* occurs much more frequently in isolates implicated in avian colibacillosis than it does in fecal *E. coli* of healthy birds. Recent findings in our lab have localized *iss*, along with several other genes thought to be involved with avian *E. coli* virulence, to a single transmissible R plasmid in an avian isolate. In the present study, an *iss*-containing fragment of this plasmid that was obtained by digestion with *Eco*RI was subcloned, sequenced and analyzed in order to provide more insight into the virulence mechanisms of avian *E. coli*. Procedures involved in the sequencing of this plasmid and implications of the results will be discussed.

Production of Monoclonal Antibodies Against Avian *Escherichia coli* Iss

Aaron M. Lynne
Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND
Co-authors: Steven L. Foley and Lisa K. Nolan

We have found that the presence of *iss* DNA sequences is strongly correlated with *E. coli* implicated in avian colibacillosis, making *iss*, and the protein it encodes (Iss), candidate markers of avian *E. coli* virulence. As part of an overall effort to explore potential colibacillosis control strategies, monoclonal antibodies (Mabs) to Iss have been generated by immunizing mice with calmodulin binding peptide-Iss (CBP-Iss). Hybridomas were produced by the fusion of spleenocytes from the hyper-immune Balb/C mouse and SP2/O mouse myeloma cells. *E. coli* isolates known to possess or lack *iss* sequences have been screened using the Mabs in ELISA and Western blotting protocols in order to determine the potential utility of these Mabs in detection of virulent avian *E. coli.*

Adaptation of Virulent Serotype I Infectious Bursal Disease Virus to Avian Macrophages

Hayet Abbassi
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul, MN
Co-authors: H. Gerbyshak-Szudy, H.Y. Yeh and J.M. Sharma

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speculate that differences in pathogenicity may not mainly be due to local events in the bursa but may be depend the ability of the virus to affect the systemic immune system.

The Effect of Subclinical Infectious Bursal Disease on Iron, Copper, and Zinc Levels in Organs During the Acute Phase Response

Craig S. Blackmore
University of California-Davis
Co-authors: Patricia S. Wakenell, Kirk C. Klasing and Donald B. Link

Fifty (50) SPF chicks maintained together up to one (1) week of age when they were separated. Twenty-five (25) were challenged with standard IBDV challenge virus and twenty-five (25) were left as controls. Five (5) birds were randomly selected from the challenge and control groups for liver, spleen, bursa, pancreas and serum samples to be taken at each of five (5) specific time periods 3 days, 6 days, 9 days and 12 days post infection. The samples were flash frozen using liquid nitrogen and stored at -20°F. The analysis was performed using atomic absorption to find the levels of iron, copper and zinc in each of the organs and serum at each specific time during the acute phase reaction for challenged as compared to control birds.

Investigation of Sub-clinical Infectious Bursal Disease in Broiler Farms with a History of Production and Disease Problems

Nathaniel L. Tablante
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland Campus
Co-authors: Daniel A. Bautista, Fidelis N. Hegngi, Conrad Pope and Chinta Lamichhane

Five broiler flocks on farms with a history of production and disease problems and one apparent healthy (control) flock on a farm with no history of production and disease problems were monitored over one production cycle through field observations, serology, histopathology, and monoclonal assay (antigen-capture ELISA). Four of the problem flocks showed a spike in IBD titers at 3.5 weeks of age. The standard strain of IBD virus was identified in two of these flocks, GLS in one flock, and both Delaware E and Standard strains in another. The control flock showed normal and stable IBD antibody titers, tight CV's, and absence of IBD infection throughout the growing period indicating that good flock uniformity, high and uniform IBD titers, and good management contribute to good flock performance.

Recombinant, Attenuated IBDV Vaccine Protects Against Classic and Variant Strains

Meihong Liu
Center for Agricultural Biotechnology, University of Maryland Biotech Institute
Co-authors: Meggin Brandt, Yi Liu, Gerard Edwards and Vikam Vakharia

To develop an attenuated, multi-spectrum vaccine candidate that can protect against classical and variant strains of infectious bursal disease virus (IBDV), we constructed a chimeric cDNA clone of segment A of the D78 and GLS strains. Using the cRNA-based reverse genetics system, we generated a recombinant virus after transfection in Vero cells. A panel of IBDV-specific monoclonal antibodies was used to characterize the recombinant virus and its replication kinetics was compared with that of the parental D78 strain in vitro. Virus deficient in the expression of VP5 nonstructural protein (NS) grew to slightly lower titers than D78 virus and exhibited decreased cytotoxic and apoptotic effects in cell culture. In addition, this virus failed to induce any pathological lesions in the bursa of inoculated chickens. To evaluate efficacy of the recombinant IBDV vaccine, we inoculated 3-week-old chickens with this virus and then challenged them with STC and variant GLS viruses. Based on histopathology and serology results, complete active protection was afforded by a single dose of the recombinant virus vaccine against both classic and variant IBDV strains.

DNA Vaccination Against Classical and Variant Infectious Bursal Disease Virus in Chickens

Ching Ching Wu
Purdue University, West Lafayette, IN
Co-authors: Tsang Long Lin, Hua Chen Chang and Ming Kun Hsieh

Two plasmids containing large segment gene of variant E (VE) infectious bursal disease virus (IBDV), designated as pVE, or large segment gene of standard challenge strain (STC) IBDV, designated as pSTC, were constructed and used as DNA vaccine. One-day-old chickens were intramuscularly injected with individual plasmid or both plasmids and subsequently, boosted two times at weekly interval. Chickens were challenged with STC or VE at 21 days old. Chickens received pSTC had 100% protection when challenged with STC, but only 20% protection when challenged with VE. Plasmid pVE provided 90% protection against VE and 70% protection against STC. Chickens received both plasmids have 80% protection against VE and STC, respectively. The results indicated that chickens vaccinated with plasmid pVE alone or both plasmids (pVE and pSTC) can confer adequate protection for chickens against classical or variant IBDV infection.

Site-Directed Mutagenesis of in vitro Expressed IBV Spike Glycoproteins in an Avian Cell Line

Emma D. Wade
University of Georgia, Athens, GA
Co-authors: Mark W. Jackwood and Deborah A. Hilt

The spike glycoprotein of infectious bronchitis virus, comprised of the S1 and S2 subunits, contains a number of predicted antigenic and structural regions. Specific sites will be chosen for site-directed mutagenesis and the resulting genes will be cloned into a mammalian expression vector for transfection and expression in an avian cell line. Previously characterized monoclonal antibodies will be used for indirect immunofluorescence to identify changes in conformation of the spike glycoproteins. The goal of this research is to identify areas of the spike subunits necessary for retention of native conformation and antigenicity.

Recognition of a Unique, Widely-Disseminated Infectious Bronchitis Virus Genotype in North America; Implications for Understanding the Spread of the Disease

Jack Gelb, Jr.
Department of Animal and Food Sciences, University of Delaware, Newark, DE
Co-authors: Brian S. Ladman, Peter R. Woolcock, Frederic J. Hoerr, Kalen C. Cookson, Darrell W. Trampel, Andre F. Ziegler and Brian D. Binnington

A unique infectious bronchitis virus (IBV) S1 genotype has been reported from a wide geographic area in North America. First recognized in Pennsylvania, genotype PA/1220/98 was obtained from 20-week-old commercial layer pullets with respiratory disease. Since 1998, isolates highly related to PA/1220/98 were recovered in California, Iowa, and Ontario, Canada. All of the isolates have come from layer or layer pullet flocks, many of which were located on large multiage farm complexes. In layers, egg production was decreased and accompanied by shell quality problems.

The recognition of a unique genotype unrelated to any other known IBV provides an opportunity to speculate how avian pathogens might be spread across long distances. The author will discuss potential means of transmission and input of the audience will be sought.

Infectious Bronchitis Virus – the "Cal 99" Strain

Peter R Woolcock
California Animal Health & Food Safety Laboratory System, Fresno Branch, University of California, Davis
Co-authors: Michael McFarland, Sharon Hietala and Liu-Mei Shih

Objective:
To report on the characterization and properties of the Cal 99 isolate of Infectious Bronchitis virus (IBV).

Sample population:
Broiler chicken cases submitted for necropsy to the California Animal Health & Food Safety Laboratory System.

Results and Conclusions:
In 1999 we began isolating an IBV from broilers, usually more than 40 days of age. The virus was associated with respiratory disease and increased condemnations due to airsacculitis. Isolates of this virus could not be typed with monoclonal antibodies to the Ark, Conn or Mass serotypes and by polyacrylamide gel electrophoresis and immunoblotting of the matrix proteins isolates were consistently identified as Mass-type. RT-PCR and RFLP provided variable results, ranging from untypable, Mass-type, Nebraska 95, and not amplified. RT-PCR and direct automated cycle sequencing (DACS) of the hypervariable region of the S1 gene and comparison with sequences logged in Genbank gave the closest identity as 85% to Ark 99 and Ark DPI. This isolate was named Cal 99 and the sequence was logged in GenBank. Further details of the pathology, cross protection, typing, and relationship of this strain to other IBVs will be presented.

In-ovo Vaccination for IBV using DNA vaccine: A Preliminary Study

Mazhar I. Khan
Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT
Co-authors: Jaroslaw J. Fabis and Ted Grishick

The use of attenuated vaccines poses the concern of artificially introducing the virus into a flock and provides the potential of emerging new IBV strains by either point mutation or recombination. Lately a new approach was taken and more interest has been directed towards the use of recombinant DNA vaccines, as a safer and more efficient ways of inducing immunity against diseases.

The new technology using in-ovo immunization makes DNA based vaccine even more competitive and effective against poultry disease agents. The plasmid DNA (S1 &S) vaccines were administered into the egg's albumin on 14th days of embryonating SPF chicken eggs using in-ovo technique respectively. The 7 days hatched chicks were challenged with the live pathogenic IB virus. Their immune response and pathogenicity are being characterized and these data will be presented at the meeting.

Wednesday, July 17, 2002
Session B

Chick Length Uniformity Profiles as a Field Measurement of Chick Quality?

Donna Hill
Donna Hill Consulting

Based on research and field observations of growth restriction related to incubation, crown to base of tail measurements chicks were used to develop uniformity profiles in various areas of the egg mass.

The chick length uniformity profile has show the following trends to date:

- Chick length increases with breeder flock age
- Chick length in multistage incubation in the US is larger in the summer than in the spring.
- Preliminary data suggests that chick length in a single stage incubation system is larger than in multistage incubation.
- The shortest chick length is found in hatcheries with the greatest one-week mortality problems and hatchery problems, for example, positive hatcher plenum exhaust.
- Chick and poult length varies based on location within the hatcher. This is predictable within machine types.
- In multistage incubation, the chick length of chicks hatched from the oldest breeder flocks is smaller than the prime flocks in the same hatchery.
- In the single stage system, chick length increased as flock age increased.
- The measurement appears to be valid for poults also.
- Chick length can be used as a field diagnostic tool. When the chicks that die in the first week are the same size as the average 18 day embryo, the problem is in the incubation of the embryo.

The chick length uniformity profile may be a very useful tool to monitor chick quality over time. Once the incubation quality and chick performance relationships can be evaluated seasonally, economic justifications for upgrades in hatchery equipment can be made based on performance costs.

Innovations of In ovo Technology

Marco A. Quiroz
AviTech LLC, Hebron, MD
Co-authors: Rafael S. Correa, William Samson and Erich Bevensee

The in ovo technology has been used commercially in the US poultry industry since the early 1990's. Because of all the advantages that the in ovo vaccination provides for the poultry producers, this practice has extended to many other regions of the world, like Asia, Europe, South America and the Far East.

As in all fields, the in ovo technology should also be subject to continuous improvements. With this in mind, AviTech, LLC has developed a completely new in ovo technology that brings an innovative concept to the in ovo immunization in the poultry industry.

This presentation will describe a new in ovo immunization concept. We will describe the philosophy behind it and how this concept will help maximize the Marek's vaccine stability and biosecurity during the process.

This talk will also discuss how this new technology will help reduce the potential of cross contamination and the use of antibiotics during the in ovo vaccination.

Comparison of In Ovo and Post-hatch Injection of Gentamicin Sulfate in Turkey Poults

David V. Rives
Prestage Farms, Inc.

An eight week study involving approximately three million eggs compared the Inovoject system from Embrex, Inc. to a poult injection system from Nova-Tech. The study was initiated in January, 2001. Gentamicin sulfate was injected on alternate weeks either in ovo at transfer or subcutaneously at the time of infrared beak trimming. Hatchability of injected versus non-injected eggs was monitored. First week mortality of in ovo versus subcutaneously injected poults was also monitored. Gentamicin resistance patterns of selected bacteria for the past three years will also be presented.

Experiences Raising Commercial Chickens Without the Use of Conventional Antimicroibial Interventions

Spangler Klopp
Townsends, Inc.

Raising flocks of meat type chickens requires management procedures to combat specific infectious diseases. Principle components of such programs include vaccination, proper feeding and nutrition as well as good air and litter quality. With out pharmaceutical interventions, added emphasis on all these components is required. Nonuse of such interventions is hoped to reduce the development of resistant bacteria entering the food chain. Discussion will describe procedures used to counter the use of such medications and their repercussions on the flocks of chickens as well as the cost of production and the size and general scheme of the production system.

Drug Free Chemical Free Broiler Production - Is It Commercially Feasible?

Arun Bahl
Co-author: Nino Sorgente

Poultry producers in all parts of the world are increasingly facing legislative and consumer pressures to reduce and/or eliminate the use of antibiotic growth promoters and chemicals that are related to treat human diseases. In the European Union all antibiotic growth promoters except two (avylamycin and bambermycin) are banned from inclusion in broiler feed since June 1999. As an outcome of this action poultry farmers in the European community have experienced increased problems related with clostrida, dermatitis, necrotic enteritis, and ??? (words blocked out on fax) with Clostridium perfrigens. Negative effects on performance, live weight, mortality and feed conversion ratios have been well documented.

Our data using highly purified patented naturally occurring beta-1,3/1,6-flucan molecule derived from Baker's yeast, Saccharomyces cerevisiae, yeast nucleotide extracts along with essential oils extract from oil or oregano (origanum vulgare

sppe hirtum) has given very promising results under field conditions. Field trial data will include clinical observations, mortality, feed conversions without the use of growth promotion antibiotics and or coccidiostats

The Effects of Poultry Production Units on the Air Quality of Local Environments

Meredith F. Davis
Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH
Co-author: Teresa Y. Morishita

The impact of modern commercial poultry production methods on the local environment of the units has been of great interest in the past few decades. Poultry production units have been scrutinized for violations of the Clean Air Act and the financial penalties to such production units has been substantial. Air quality issues that have been associated with this intensive form of farming include, but are not limited to, organic dust concentrations, aerosolized bacteria, and ammonia levels. The purpose of this research was to quantify the dust concentrations, determine the presence of aerosolized Salmonella and E.coli, and measure the ammonia levels inside and outside of five commercial layer houses.

Molecular Biological Tools for Routine Diagnosis and Further Characterization of Avian Poxviruses

H.M. Hafez
Institute of Poultry Diseases, Free University Berlin, Germany
Co-authors: T. Hoffmann and D. Lüschow

Fowl pox is a disease of chickens and turkeys with a worldwide distribution and is caused by a DNA virus of the genus *Avipoxvirus* of the family Poxviridae. Fowl pox virus (FPV) is the prototypical member of this genus. Other important antigenically related, but distinguishable poxviruses were recovered from various species of birds and names are given related to their hosts, such as canary pox, turkey pox and pigeon pox.

In Germany fowl pox infection have never been seen for the past 25 years in poultry farms with intensive rearing system. In the last two years a re-emerging of fowl pox outbreaks was observed in layer, broiler breeder as well as in turkey breeder flocks at different area in Germany.

The aim of the present study was to develop a sensitive molecular biological tool for routine diagnosis and differentiation of various avian poxviruses. We established a reliable poxvirus polymerase chain reaction (PCR), which confidently diagnosis poxvirus genome from original material or from infected chorio allantoic membrane (CAM) of chicken embryos. The applied primers framed a region within the FPV 4b core protein gene and were able to amplified DNA of the most known Avipoxviruses like fowlpox, turkeypox, pigeonpox, canarypox and sparrowpox. Further investigations by restriction enzyme analysis (REA) of PCR products using different endonucleases were carried out. Using this methods we were able to distinguish various species of avipox virus like fowlpox, pigeonpox or canarypox. The PCR and REA using in our laboratory are sensitive techniques for routine diagnosis and for epidemiological investigations in the future.

Diagnosis and Characterization of Avianpox Viruses from Wild Birds

Deoki N. Tripathy
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL
Co-author: T-J Kim

Poxviruses of the genus Avipoxvirus of the Poxviridae family infect both domestic and wild birds. The type species of the genus, fowlpox virus, has been characterized extensively because of its economic importance for commercial poultry. Unfortunately, such genetic, antigenic and biologic information is not available regarding other avianpox viruses. Prior to determining how poxviruses from wild birds are related to fowlpox virus, their initial isolation either in the chicken embryos or avian cell cultures is necessary. These viruses show not only differences in their replicative ability in chicken embryos but many of them can not be propagated in avian cell cultures.

A Review of *Eimeria mivati* – Edgar and Seibold

S.Fitz-Coy
Schering-Plough, Animal Health, American Scientific Laboratories, Inc.
Co-authors: M. Eckman and B. Brewer

Eimeria mivati was originally isolated from chickens in the state of Florida in November 1959. In 1964, Edgar and Seibold named and described *E. mivati* as a new pathogenic species of coccidia for chickens. The years following, *E. mivati* was identified in 27 states, Canada, Europe, and Lebanon.

In late 1960's and early 1970's, several researchers believed that *E. mitis* was a sub-population of *E. acervulina*. By the 1980's, some confusion about *E. mivati* began. This led to the notion that *E. acervulina* and *E. mivati* are one of the same. The current belief by some is that *E. mivati* is a sub-population of *E. acervulina*.

In the late 1950's and early 1960's, Edgar and Seibold used immunization and cross immunization studies to demonstrate that the organisms named and described as *E. mivati* were different from the known species of chicken coccidia. Data from those studies were not published.

Edgar and Seibold showed the initial infection in the upper small intestine, then as the infection aged it migrated towards the large intestine. The oocyst size and shape indexes of *E. mivati* most often overlap those of *E. mitis* and *E. necatrix*, and less often those of *E. acervulina, E. praecox* and *E. hagani.*

Occasionally a few oocysts are obtained from commercial chickens that appear to be similar to those described for *E. mivati.*

DNA Vaccination Strategy Against Avian Coccidiosis

Hyun S. Lillehoj
Parasite Biology, Epidemiology and Systematics Laboratory, USDA, ARS
Co-author: Wongi Min

Avian coccidiosis, an economically important disease for the poultry industry, is caused by intracellular protozoa belonging to several species of the genus *Eimeria*. Infection with *Eimeria spp.* results in extensive destruction of the intestinal epithelium accompanied by severe depression in body weight gain, reduced feed efficiency and intestinal shedding of parasite oocysts. Application of antigen-encoding DNA plasmids as vaccine has been shown to successfully induce protective immune responses against a variety of infectious diseases. Since the emergence of gene-based immunization methodologies, various strategies have been used to increase the efficiency of DNA vaccination. In this report, new results from our DNA vaccination studies will be addressed.

A Comparison of Coccivac-B to an Anticoccidial Program on Pigmentation of Broiler Chickens

Greg F. Mathis
Southern Poultry Research, Inc.
Co-author: Charles Broussard

The objective was to compare the effect on pigmentation of broilers vaccinated with Coccivac-B (nonmedicated feed) or Salinomycin 60 g/ton. A liquid xanthophyll supplement was added at a rate of 12 mg/ton grower and 15 mg/ton finisher. Treatments had seven replications. Fecal oocyst counts confirmed that the test system was adequate and the coccidia were at an expected level. Using the Roche Color Fan, on Days 40, 42, and 44, male and female birds were shank pigmentation scored. Scores were comparable to field shank scores. No significant difference in degree of pigmentation was observed between the Coccivac-B and Salinomycin.

Multifocal Necrotic Duodenitis: A Novel Disease of Commercial Laying Hens

Eva A. Wallner-Pendleton
Animal Diagnostic Center, Pennsylvania State University, University Park, PA
Co-Authors: Eric Gingerich, Robert Norton, Patricia Dunn and David Kradel

Multifocal necrotic duodenitis has been observed in Pennsylvania commercial laying flocks since 1996. Unfortunately, the cause of this disease has been notoriously difficult to determine. Affected birds show no outward clinical signs. There is a drop in egg case weight. Necropsy of infected chickens demonstrates green oval foci with yellow caseous centers. On microscopic examination of these foci, intense heterophilic infiltrates are observed in the lamina propria. Necrosis is seen in the upper third of the villi, with large numbers of thin, long, gram-stain variable bacteria arranged in parallel formation. Until recently, bacterial isolation results have not yielded any one specific organism. Attempts to reproduce the disease with intestinal homogenates taken from sick birds have been unsuccessful. The focus of this presentation is to discuss new microbiological findings using strict anaerobic techniques. Several flocks are being followed epidemiologically by a team of veterinarians, nutritionists, avian physiologists and microbiologists. Upper gastrointestinal pathophysiologic factors and diet will also be presented.

Enteritis of Clostridial Etiology in Cage Layers with High Fly Population

A. Singh Dhillon
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Co-authors: L. H. Lauerman, Parimal Roy; Dennis Schaberg; Daina Bandli and Sylvia Weber

High mortality was reported in a new high-rise lay farm, with bird capacity of 180,000. The gross and histopathologic lesions were consistent with clostridial enteritis. *Clostridium perfringens* was isolated from the affected intestine. In addition, *Escherichia coli*, and on occasion *Pasteurella hemolytica*, was isolated from the liver and peritoneal surfaces. Mild coccidiosis infection was also present. Five houses were affected but mortality was high in one house. The four-week mortality was 6.56 percent with a loss of 10,898 chickens. The four-week mortality rate in the other houses was moderately high. Amprolium was used to treat coccidiosis, and household bleach was added to water at a dilution of one part of bleach to 1,040 parts of water to reduce bacterial infection.

The fly population was out of control and *C. perfringens* was isolated from the alcohol-washed macerated flies. The serum samples of affected birds and the macerated alcohol-washed and clarified fly extract caused illness, paralysis and death in the inoculated mouse. The *C. perfringens* isolated from the chicken and flies was identified as *Clostridium perfringens* type A by PCR. The straw was added and mixed in with litter. As a result, the litter temperature increased that led to a reduced fly population and reduction of mortality to normal.

Poster Program

Poster 1

The Impact of the 1999-2001 Italian Avian Influenza Epidemic on the Diagnostic Laboratory

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The paper summarizes the diagnostic activity performed by the Italian National Reference Laboratory for Newcastle Disease and Avian Influenza, during the 1999-2001 avian influenza epidemic, which ultimately resulted in the death or culling of over 13,000,000 birds. The number of serological and virological diagnostic tests performed during the epidemic and following the implementation of the vaccination policy are reported.

Poster 2

A Plasmid-based Reverse Genetics System for Avian Influenza Virus

Chang-Won Lee
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Co-author: David L. Suarez

To generate avian influenza (AI) virus from cloned cDNA, we constructed 12 plasmids that contains the viral sequence of highly pathogenic AI virus. Four protein expression plasmids were constructed by inserting the open reading frames of PB2, PB1, PA and NP proteins downstream of the CMV promoter in the pCIneo plasmid. Eight transcription plasmids were further constructed by inserting viral genomic sequences between the human RNA polymerase I promoter and the mouse RNA polymerase I terminator sequence in the pHH21 vector. The suitability of these constructs for the rescue of virus will be assessed with following experiments. First, the hemagglutinin gene in the pHH21 vector (DK-HA-pHH21) will be transfected, followed by infection with low pathogenic AI helper virus, and the transfectant virus will be selected by plaque formation without trypsin. Second, viral RNPs will be reconstituted in tissue culture cells from four expression plasmids, and the synthetic HA gene into a recombinant influenza virus will be rescued following infection with helper virus. Finally, AI virus will be generated entirely from 12 cloned cDNAs.

Poster 3

Safety of Ketamine - Xylazine Mixture for Anaethesia of Some Free Living and Caged Birds

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Co-author: S. Mousa

Summary

Efficacy and safety of different doses of ketamine – xylazine mixture were tested for pigeon, doves and budgerigars after intramuscular injection. Doses of 0.5 – 1.0 mg Ketamine and 0.1 – 0.2 mg xylazine was the proper dose for budgerigars, while 5 mg ketamine and 1 mg xylazine was the suitable dose for pigeon and doves.

Long term safety was proved by clinical observation, some surgical operations as well as parameters of liver and kidney function tests.

Poster 4

Evaluation of Growth Enhancement Antibiotics and Possible Alternatives in Broiler Diets

Tim E. Cherry
SFASU
Co-author: Copie Roberts

This study has a two-fold objective: First to determine if performance on the alternative additives was equal to or better than the growth enhancing antibiotics as determined by feed conversion, weight gain, and yield study. Secondly does the ionophore antibiotic interfere with the growth enhancement activity of the alternatives? Treatment groups were as follows:

Treatment	Starter Feed	Grower Feed	Withdrawal Feed
1	Bacitracin/Salinomycin	Bacitracin/Salinomycin	Bacitracin
2	Bambermysins/Salinomycin	Bambermysins/Salinomycin	Bamberysins
3	No antibiotic/Salinomycin	No antibiotic/Salinomycin	No antibiotic
4	Syner-Max 1%/Salinomycin	Syner-Max 1%/Salinomycin	Syner-Max 1%
5	Syner-Max .5%/Salinomycin	Syner-Max .5%/Salinomycin	Syner-Max .5%
6	Bambermysins/Coccivac	Bambermysins	Bambermysins

7	Syner-Max 1%/Cocivac	Syner-Max 1%	Syner-Max 1%
8	Syner-Max .5%/Cocivac	Syner-Max .5%	Syner-Max .5%

Results are reported on the poster.

Poster 5

Tracking the Claim "If There's One Sick Bird the Entire Flock is Treated"

Eric C. Gonder
Goldsboro Milling

The Food & Drug Administration included this claim in an early 2001 FDA Consumer article. It evolved from a 1994 statement by a senior Public Health Service official, through a series of docket responses, letters, and news stories.

FDA Veterinarian reprinted this article. Later, the Center for Veterinary Medicine stated that no information supporting the claim could be produced. FDA retained the original article on its web site through December, 2001 after printing a brief correction in the November/December 2001 FDA Consumer. As of November 8, 2001, FDA "considers the matter closed".

Correspondence between FDA, CVM, and the author is included.

Poster 6

Comparison of Antimicrobial Susceptibility Patterns in the Same *E. coli* from Field Cases Taken from Blood Agar Plates Versus MacConkey Agar Plates

G. Lynne Luna

Determining the susceptibility of bacteria to certain antimicrobials is very important in deciding which medication to use when treating a diseased flock of chickens, especially with *E. coli* air sac infection. Diagnostic labs are pressed to turn out results rapidly so that appropriate information is available for timely and effective treatment of the birds, thereby decreasing mortality and morbidity in the flock. Because of this time factor, most labs that isolate *E. coli* will pull it directly from the initial isolation on a MacConkey agar plate to be sure the proper bacteria is attained for susceptibility. The instructions for the susceptibility test state, however, that the isolate should be pulled from a multi-purpose agar such as TSA or Blood. This is often impractical because general purpose plates often contain more than one type of bacteria which then need to be separated to attain pure cultures for the test. Because of the increasing importance of bacterial antimicrobial susceptibilities in poultry as a human health issue, the validity of the "short-cut" results may be questioned when looking at resistance patterns. This study was done in order to determine if susceptibilities for *E. coli* isolated from chickens pulled off of MacConkey agar will give similar results as those from Blood agar.

For this study, swabs from birds with suspected *E. coli* infections were plated to both blood and MacConkey agars. Colonies of *E. coli* from each type of agar were pulled and used in the susceptibility testing utilizing the Kirby-Bauer method. The antimicrobials utilized were sulfamthoxazole trimethoprim, penicillin, gentamicin, erythromycin, tetracycline, ceftiofur, enrofloxacin, and clindamycin. The zones of non-growth were then compared between each case for each of the antimicrobials.

After comparison of 16 *E. coli's* from field cases, initial review of the data suggests that when looking strictly at "Resistance", "Intermediate", and "Sensitive" results, blood plate and MacConkey agar plates give similar results. Some variation did occur with gentamicin and ceftiofur but these were not great and the variability was between "resistant" and "intermediate" or "sensitive" and "intermediate". None of the tested isolates differed between "resistant" and "sensitive" in this study. Actual diameter measurements however, were fairly inconsistent, being off by one to several millimeters in some cases, potentially giving a false impression of drug efficacy. Statistical analysis has not yet been done as of the time this abstract was due, and more bacterial isolates will be added to this study.

Poster 7

Vertical Transmission of Campylobacter Species in Commercial Broiler Chickens

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A survey study was carried out on 30 chicks at ages ranged from day of hatch to 7 days old; in an attempt to determine the incidence and type of *Campylobacter* isolates in cecal and ileal contents. We used brain heart infusion broth, tryptic soy broth (each with 0.6% yeast) and Blood agar plates as solid media. Cultures were incubated under 2 different microaerobic conditions for 24 to 72 hr at 37ºC. Results showed that no *Campylobacter* isolate was detected by any of the above culture methods. When the DNA was extracted from ileal and cecal contents of day of hatch, *Campylobacter species* were detected by PCR but *no Campylobacter jejuni* or *Campylobacter coli* were identified by species specific PCR. Results suggest that epidemiology *of Campylobacter* transmission in broiler chicken might involve vertical transmission.

Poster 8

A Real-Time Quantitative PCR (qPCR)-Based Serum Neutralization Test for Detection and Titration of Neutralizing Antibodies to Chicken Anemia Virus (CAV)

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Detection and titration of neutralizing antibodies to CAV has relied on tedious, time-consuming passaging of infected cells, because CAV replicates in culture only in lymphoblastoid cell lines and thus does not generate plaques. We have developed a rapid method, in which CAV in infected cells is quantitated by qPCR 3-4 days p.i., without the need to passage the cells. Sera from three broiler chickens immunized with a commercial CAV vaccine were used to develop the assay. These sera had similar S/N values (ranging from 0.47- 0.51), when tested at a 1:10 dilution using a commercial ELISA kit. We determined virus neutralization titers of these sera using two different CAV-susceptible cell lines (MSB-1 and CU-147) by the conventional method of passaging cells infected with 10^4 $TCID_{50}$/well, and by qPCR-based methods using cells infected with 10^4 or 100 $TCID_{50}$/ well in 6-well or 96-well plates. Because not everyone has access to qPCR instrumentation, we have also adapted the method to conventional PCR. The three positive sera exhibited virus neutralization activity at dilutions ranging from 1:10 to 1:320 by the various assays. Negative sera showed no virus neutralization activity by any assay. Although the virus neutralization titers obtained differed somewhat depending on the assay conditions used, the relative order of the titers obtained from the three positive sera was the same for all assays. We found the qPCR-based assays to be more sensitive for detection of neutralizing antibody than the conventional assay based on passaging infected cells.

Poster 9

Generation of Infectious Clones of Chicken Anemia Virus (CAV) Directly from Clinical Specimens by PCR

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Complete genomes of two different CAVs were generated from cloned overlapping PCR fragments produced directly from clinical specimens, without prior isolation of the virus. After electroporation of the genomes into CU-147 lymphoblastoid cells, cultures failed within 9 days, and the medium of the cultures contained up to 5 X 10^6 $TCID_{50}$ CAV, confirmed by PCR. Identical electroporation of MSB-1 cells failed to destroy the cultures even after 57 days, although MSB-1 cells were susceptible to low doses (1000 $TCID_{50}$/ml) of at least one of the viruses generated by electroporation of CU-147 cells. The replication of these viruses in MSB-1 and CU-147 cells at 37˚, 39˚, and 41˚ was characterized. As expected, CU-147 cultures infected with 1000 $TCID_{50}$/ml of one of our CAVs were destroyed more rapidly (7-10 days) than infected MSB-1 cultures (14-23 days). In both cell lines, we found cultures were destroyed by CAV infection (1000 $TCID_{50}$/ml) more slowly when grown at 37˚ than at 39˚ or 41˚. MSB-1 cultures were destroyed more rapidly by CAV infection at 39˚ than at 41˚, while no difference between these two temperatures was noted for CU-147 cells. Although low passage MSB-1 cultures infected with 1000 $TCID_{50}$/ml were destroyed more rapidly than high passage MSB-1 cultures, we detected no difference in rate of virus replication in one-step growth curves at different MSB-1 cell passage levels. The slower destruction of high passage MSB-1 cultures may be related to the faster replication of MSB-1 cells, which we noted at higher passage levels.

Poster 10

Pathological Manifestations of Pasteurella Multocida Infection in Broiler Chickens

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Summary
Pasturella multocida (PM) was incriminated in several cases of disease affections in broiler flocks. Affected birds ranged from 4 – 7 weeks of age. Morbidity rate ranged from 5 – 40%, while mortality rate was 1 – 6% within one week. PM was isolated from cases showing head and facial swelling with accumulation of gelatinous material in subcutaneous tissue of the head Other flocks showed sudden death with lesions of general congestion and petichae on serosal membranes. Some positive flocks showed respiratory distress with lesions of general congestion and hemorrhagic tracheitis. Cases showing arthritis showed lameness and accumulation of serous to caseous exudates in joints. PM was identified biochemically, by laboratory animal inoculation and serologically. Some clinical manifestations were successfully reproduced. Isolates were usually sensitive to gentamycin, chloramphenicol, streptomycin lincospectin and quinolones.

Poster 11

Dietary Modulation of Host Protective Immunity Against Avian Coccidiosis

Hyun S. Lillehoj
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Avian coccidiosis is a major parasitic disease of poultry of economic significance. Currently, drugs and live parasite vaccines are two main ways to control coccidiosis. Due to increasing problems with the prolonged drug usage, novel control strategy is needed for effective control of coccidiosis in chickens. Recent evidence that various dietary supplements can influence host immunity against various diseases prompted us to investigate the role of dietary factors on coccidiosis. This presentation will review our latest studies on the role of vitamins and probiotics on host mucosal immune response against coccidia parasites.

Poster 12

Coronavirus in Turkeys; Are they Turkey Coronavirus or Infectious Bronchitis Virus?

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The original turkey coronavirus (TCV) described in 1951 causes Bluecomb disease, and is similar to bovine coronavirus, a group II coronavirus. Recent coronavirus isolates associated with enteritis in turkeys appear to be genetically similar to a group III coronavirus, infectious bronchitis virus (IBV), which is unique from all other coronaviruses. In this study we examine the gene order of these recent coronaviruses, which suggests that they fall into Group III. In addition, we will present the phylogenetic relationship of those viruses to other coronaviruses. This information is important because if indeed these new isolates are group III coronaviruses similar to IBV, they may have the potential for multiple serotypes and for causing disease in chickens.

Poster 13

Complement Resistance, As Determined By Flow Cytometry, Of Avian *Escherichia coli* Isolates

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Co-authors: Catherine W. Giddings and Shelley M. Horne

Over 500 *Escherichia coli* isolates of sick and healthy birds were classified according to complement resistance, serotype, associated disease syndrome, and antimicrobial resistances. This characterization was undertaken in order to determine if a certain pattern of traits is associated with the complement resistance and virulence of avian *E. coli*.

Poster 14

Development of A Multiplex PCR Protocol to Discern Virulent from Avirulent Avian *Escherichia coli*

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Summary
Colibacillosis is a major problem in the poultry industry. Control of this problem has been hindered because there is no single, easily identifiable characteristic associated with disease causing avian *Escherichia coli*. In this study an attempt was made to develop a Multiplex PCR protocol to facilitate identification of *E. coli* capable of causing avian colibacillosis. This PCR protocol amplifies four genes including *tsh*, which encodes the Temperature Sensitive Hemagglutinin, *iss*, the increased serum survival gene, *cvaC*, the structural gene of the ColV operon, and *iucC*, a gene of the aerobactin iron transport operon. These genes were chosen because of their association with avian *E. coli* virulence and their possible linkage to the same plasmid. Twenty well-characterized *E. coli* isolates, ten from sick birds and ten from apparently healthy birds, were chosen for this study. When the Multiplex PCR protocol was performed upon the isolates, 80% of the isolates from diseased birds contained at least three of the four genes while the remaining isolates contained two of the four genes. One of the ten isolates from apparently healthy birds contained all four genes. This isolate, when tested in a chick embryo lethality assay, was found to be virulent. The remaining nine isolates from apparently healthy birds contained no more than one of the genes. In general, it appears that the possession of two or more of these genes was a strong predictor of virulence among these isolates.

Poster 15

Educating Service Technicians through Poultry Workshops

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A series of workshops was initiated in 1997 for the Mississippi poultry industry. The purpose of these day-long events is to provide industry personnel with knowledge of "real world" experiences in areas that will enhance their productivity. Several workshops have been held for service technicians from around Mississippi and portions of Alabama. All poultry complexes within Mississippi have participated. Such topics as principles of disease management, communication skills, bio-security, principles of ventilation, and fertility and hatchability have been addressed. Directly or indirectly, these workshops have a positive influence on the poultry industry personnel, the poultry companies, and the poultry growers.

Poster 16

A Web-based Biosecurity Training Course for the Poultry Industry

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A web-based biosecurity training course was developed by the University of Maryland through a distance learning grant. The course is aimed at poultry industry personnel who need training on biosecurity and poultry disease prevention but cannot attend formal classroom-type sessions. The domestic and international expansion of the commercial poultry industry has led to an increased movement of personnel, vehicles, equipment, and supplies. These movements greatly increase the potential for disease outbreaks and have further heightened the importance of biosecurity--a system aimed at preventing disease outbreaks in poultry flocks. A web-based user-friendly course on biosecurity will certainly benefit local and international poultry industry personnel and help promote poultry health and disease prevention in the U.S. and throughout the world.

Poster 17

Characterization of Anaerobic Bacteria Associated with Duodenal Ulcers in Commercial Laying Hens

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A syndrome occurring in commercial laying hens has been investigated for the last five years. Birds experiencing the problem drop in weight and upon necropsy gray colored ulcers are consistently observed in the duodenal portion of the intestine. Histological examination of the lesions has consistently revealed large gram negative to gram variable rods, which previously remained unculturable. Using specialized anaerobic techniques, bacteria morphologically matching the histological descriptions have been isolated. The physiological characteristics of the bacteria will be described in detail. Other bacteria isolated from affected regions of the intestine will also be discussed.

Poster 18

The Effects of *Tetratrichomonas gallinarum* Infection in Turkeys

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Fulminating cases of *Tetratrichomonas gallinarum* infection are often observed in cases of turkey poult enteritis and/or poult enteritis and mortality syndrome. The objective of this study was to characterize the production and physiologic effects and tissue distribution of a *T. gallinarum* infection in turkeys. A virus free *T. gallinarum* inoculum was administered to 1-week-old turkey poults, with PBS administered to controls. The two ml inoculum contained 2.0 X 10^2 protozoa.

No significant effects were observed in feed intake, body weight gain, and feed conversion following 2 weeks of infection. At three weeks of age, blood samples were collected from all poults and a complete clinical chemistry blood panel was examined for alterations in normal physiology. Statistical differences ($P < 0.05$) between controls and inoculated poults were noted for Na, Cl, Ca, P, and ALT. While statistical differences were evident, the magnitude of these differences was small and all mean values were within normal range for turkeys. No differences between treatments were noted for glucose, creatinine, total protein, K, AST, ALKP, GGT, CK or uric acid.

Mucosal scrapings of cecum and ileum of all poults on experiment were examined microscopically. All poults in the control group were negative for *T. gallinarum*. On a scale of 1 to 5 (1 negative, 2 indicating 0 to 1 protozoa per high power field (HPF), 3 for 1 to 10 protozoa per HPF, 4 for 10 to 100 protozoa per HPF, and 5 greater than 100 *T. gallinarum* per HPF) the inoculated poults had a mean cecal parasitemia score of 4.72 indicating a severe *T. gallinarum* infection. The mean ileal parasitemia score for inoculated poults was 1.25, which indicates that three of four poults were negative for ileal protozoa. Likewise, histopathology revealed that 96% of the inoculated poults had a moderate or severe *T. gallinarum* infection of the cecum and 16% had a mild infection of the ileum. All sections of duodenum and jejunum from inoculated poults were negative for *T. gallinarum* as were all intestinal specimens from uninoculated poults.

Given these results one can conclude that *T. gallinarum* primarily colonizes the cecum of infected poults, and while tremendous numbers of protozoa may be noted on cecal mucosal scrapings, the parasite does not appear to cause any significant production effects in turkeys.

Poster 19

Role of CPD-Photolyase, and Acidic-Type Inclusion Body Protein in Maintaining the Infectivity and Environmental Persistence of Fowlpox Virus

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Fowlpox is one of the major diseases afflicting poultry. Despite continual vaccination with attenuated strains of the causative agent, fowlpox virus, this disease remains endemic throughout the world. One possible explanation for its resurgence is that

virulent fowlpox virus is probably endowed with the ability to produce a plethora of molecular factors that enable it to remain viable for extended periods of time. In this regard, desicciated scabs released from virus-infected chickens are known to act as havens for this pathogen. Though the molecular mechanisms underlying this phenomenon are not completely understood, we have recently identified two fowlpox virus genes whose products are likely to be involved. The first is a novel DNA repair enzyme, CPD-photolyase, whose elimination renders extracellular virus particles more sensitive to ultraviolet light exposure. The second is a protein involved in the formation of acid-type inclusion bodies and presumably protects encompassed virions from physical stress. Currently, the role of both these proteins in regards to fowlpox virus pathogenesis is being investigated.

Poster 20

Genetic Approaches Toward Characterization of Avian Poxviruses

Tae-Joong Kim
Co-authors: William Schnitzlein and Deoki Tripathy

Recently, we have isolated strains of avianpox viruses from a variety of wild birds. In order to genetically characterize these viruses rapidly we have designed degenerative PCR primer sets based on genes that are conserved in the genomes of the avian poxviruses, fowlpox virus and condorpox virus. The later was chosen since it represents a new species in the avianpox virus genus. The results of this study will assist not only in the rapid diagnosis of avianpox viruses but also in their genetic differentiation from fowlpox virus, a common pathogen of commercial poultry.

Poster 21

Evaluation of a Recombinant Vaccine for Protection Against Fowlpox in Chickens

Pratik Singh
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Co-authors: William M. Schntizlein and Deoki N. Tripathy

We have previously described the presence of reticuloendotheliosis provirus (REV) integrated in the genome of fowlpox virus (FPV) isolated from outbreaks in vaccinated chicken flocks. In order to develop an effective vaccine, we have generated a recombinant FPV in which the entire REV provirus sequence has been deleted. Currently, the potential of this recombinant as a vaccine is being evaluated in chickens.

Poster 22

Field Evaluation of a Novel Bivalent Vaccine Against Infectious Bursal Disease (IBD) and Newcastle Disease (ND) by Mixing Viruses and Antibodies Contained in Hyperimmune Egg Yolk

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Summary
A bivalent vaccine against Infectious bursal disease (IBD) and Newcastle disease (ND) was prepared by mixing viruses with antibodies contained in hyper immune-egg yolk. Experiments were conducted to test the efficacy of the vaccine in presence of maternally derived antibodies The vaccine was evaluated after injection of one-week old commercial chicks by subcutaneous route.

Serum samples were collected weekly and subjected to HI and Elisa tests. The vaccine initiated high immune response as measured by immunogenicity criteria of HI test for ND, Elisa test for IBD and challenge with either ND or IBD viruses. HI titers and Elisa titers for IBD reached its peak at 2 weeks p.v. and lasted till 6 weeks of age. Chicks were protected from challenge with either NDV or IBDV.

Poster 23

Identification of Multiple Genetic Infectious Bursal Disease Virus Populations (Quasispecies) in Commercial Vaccines following Plaque Purification

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Co-author: Daral J. Jackwood

Several commercially used infectious bursal disease (IBD) vaccines were selected for their ability to propagate in cell culture. Real-time RT-PCR was performed to confirm the presence of IBDV quasispecies in the selected vaccines. After inoculation of these strains into cell culture and plaque purification, the viral RNA was extracted. Real-time RT-PCR was then used on virus from these plaque purifications. The results indicate that the majority of the plaque purified viruses still had multiple genetic subpopulations or quasispecies.

Poster 24

Genetic Changes of Infectious Bursal Disease Virus Passaged in Vero Cells

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Co-authors: Soo Joung Kim and Dong Woo Lee

Infectious bursal disease virus (IBDV) causes an acute, highly contagious immunosuppressive disease in young chickens. Field IBDVs were isolated from IBDV suspected commercial chickens in Korea. Previous study revealed that IBDV field isolates were virulent or very virulent IBDVs. The isolates were passaged three times in chorioallantoic membrane of specific pathogen free embryonated chicken eggs and four times in Vero cell. After passage, viral RNAs were isolated and purified to determine the genetic changes. The hypervariable region of VP2 gene were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). To confirm the genetic changes, PCR products were cloned and sequenced. By sequencing analysis, the passaged IBDVs were similar to IBDV vaccine strains. Two serines in the serine-rich heptapeptide (residue 326-332) were changed into other amino acids which were similar to vaccine strains.

Poster 25

Proventriculitis in Broiler chickens: Effects of Immunosuppression

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Co-author: Tom P. Brown

Proventriculitis in broilers causes carcass condemnation due to contamination when swollen proventriculi tear during evisceration. Infectious Bursal Disease Virus (IBDV) has been implicated as the cause of proventriculitis, but our data show that IBDV does not directly cause this lesion (Pantin, ACVP 2001). This study focuses on immunosuppression and its effect on the incidence of proventriculitis. Immunosuppression will be induced in broilers using chemicals (cyclophosphamide) or virus (IBDV). Both groups will be exposed to diseased proventricular homogenate. Incidence of proventriculitis in these groups will be compared to that produced by homogenate exposure in immunocompetent broilers.

Poster 26

Genetic Variations of Avian Infectious Bronchitis Virus Isolates in Japan

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To investigate genetic variations of avian infectious bronchitis virus (IBV) in Japan. 125 IBVs which were isolated during the period from 1988 to 2001 were analyzed. A 400 base pair fragment of the S2 gene of each isolate was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) and characterized by restriction fragment length polymorphisms (RFLP)[1]. The isolates were classified into 19 genotypes and the majority of the genotypes were M-1 [2] (29.6%), Y-4 (18.4%), BN (15.2%) and C-78 (12.8%) types. Among these, the M-1, Y-4 and C-78 types have been isolated since 1988, but the BN type isolation has been made from 1997 onward. The restriction enzyme digestion profiles of these genotypes

were far different from those of viruses which belong to Massachusetts or Connecticut serotype. The variations in the IBV genotypes will be discussed together with observations on the serological variations.

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Poster 27

Comparison of the Molecular Typing of Infectious Bronchitis Virus Variants by the Restriction Fragment Length Polymorphism Assay and Phylogenetic Analysis of S1 Protein Gene

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Infectious bronchitis virus (IBV) isolated from case submissions show genetic variability at the S1 protein that can result in serotypic variation, making control of IB through vaccination difficult. To design a vaccination program it is important to identify the serotype of the IBV challenge strain. Since 1996, the reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) IBV typing assay developed by Kwon, M. Jackwood, and Gelb (Avian Dis. 37:366-374, 1993) has been successfully used for deducing serotypes of IBV case isolates in our laboratory. The S1 gene of IBV is amplified by RT-PCR and the serotype is initially deduced from the RFLP patterns. Any isolate that shows a different RFLP pattern from reference strains is called an RFLP variant. Twenty-four RFLP variants isolated from chickens among six states (AL, AR, CA, IA, IN, NC) from 1997 to 2001 were assigned to four groups by RFLP patterns: Group 1, Arkansas-like (12 isolates); Group 2; California-like (4); Group 3, PA/1220/98-like (7); and Group 4, Conn/Mass/F1/JMK-group (1). These RFLP variants were further analyzed for S1 gene sequencing. For 23/24 variant isolates, the serotype deduced from RT-PCR/RFLP matched the serotype deduced from phylogenetic analysis of the S1 gene sequence. Although classical serotyping of IBV isolates remains desirable, the molecular typing provides applicable information. For some isolates in the Arkansas-like group in this study, the application of an Ark serotype vaccine based on the molecular data prevented recurrence of the disease.

Poster 28

Gene 5 in Turkey Coronavirus Closely Related to that of Avian Infectious Bronchitis Virus

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A fragment of genomic RNA extending from 3' end of membrane (M) protein gene to 5' end of nucleocapsid (N) protein gene of turkey coronavirus (TCV) was amplified from RNA extracted from TCV-infected turkey embryo intestine by reverse transcription-polymerase chain reaction (RT-PCR). The PCR products were cloned and sequenced. The nucleic acid sequences were aligned and analyzed. Gene 5, containing two overlapping open reading frames (ORFs), 5a and 5b, was located in between M and N genes of TCV. The overall nucleotide sequences of the amplified gene 5 fragments of TCV isolates shared 88.4 to 91.8% similarity to the corresponding regions of infectious bronchitis virus (IBV) strains. The consensus transcription-associated sequence of IBV, CTTAACAA, was also highly conserved in TCV genome at the level of nucleotide sequence and located in regarding to the initiation codons of gene 5 and N gene. The similarities between the predicted amino acid sequences of ORF 5a and 5b of TCV isolates and those of IBV strains were 85.4 to 94.0%. The results indicated that gene 5 is present in TCV and gene 5 in TCV is closely related to that of IBV.

Poster 29

Protection and Productive Parameters in Broilers Using Two Infectious Bronchitis Commercial Vaccines

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Two commercial vaccines containing the strains Ma5 and H120 were evaluated measuring the protection and productive parameters in broilers. 205 Ross 308 one day old broilers were divided in five groups of 50 birds each. The vaccinated groups received one (day 1) or two dose (day 1 and 14) of live vaccine. Two of the groups, besides the live vaccine, received an inactivated oil vaccine at day one or day 10 via subcutaneous. All five groups were challenged at day 35 of age with M41 strain of infectious bronchitis virus. Post challenge were registered mortality, clinical signs, respiratory lesions and body weight as well the ciliar activity of the tracheal epithelium.

Poster 30

Evaluation of the Possible Immunological Function of the Chicken Crop (Ingluvies)

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The study evaluated crop (ingluvies) immunity and lymphoid tissue presence in pre- and post-SE infection. Forty adult specific-pathogen-free (SPF) White Leghorn hens age 78 weeks and forty-eight White Leghorn chicks age 5 weeks were selected for the study. Both groups were orally infected with Salmonella Enteritidis, phage type 13, nalidixic acid resistant strain. The humoral immune response was monitored pre-infection and then post-infection over a four week period by assessing antibody levels against SE lipopolysaccharide (LPS) antigen in serum, bile, crop, and alimentary tract by Enzyme Linked Immunosorbent Assay (ELISA). The presence or absence of lymphoid tissue was evaluated in excised, formalin-fixed crop tissues by routine Hematoxylin and Eosin (H&E) staining. Crop serial tissue sections were also used for immunohistochemistry (IHC) for the detection of the general leukocyte cluster of differentiation 45 (CD45) cell surface antigen. Each week cecal contents were cultured to assess SE levels.

Results indicated an increase in crop IgA levels and prominent lymphoid structures post-SE infection. Crop lymphoid aggregates were identified by routine H&E and CD45 positive cells were detected by IHC. These aggregates increased in size and number by week 2 post-SE infection and remained prominent though week 4 PI. Crop lavage anti-SE IgA antibody levels peaked at week 3 and week 4 for hens and chicks respectively.

Poster 31

Effect of Prior Exposure on Immunologic Response and Cellulitis Lesion Formation in UCD 003 Chickens

Joan Jeffrey
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Poster 32

Case Report: Impact of Electrical Wiring on Broiler Breeder Egg Production

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A farm was constructed to company specifications in the early 1990's to house broiler breeders. The initial flock's performance was acceptable, but subsequent flocks have not met expectations in spite of the grower's or company's efforts. Although the grower had installed the required lights, evaluation by another electrician (eight years later) revealed that the wire used in these houses was actually too small. The grower was faced with the expense of re-wiring or installing a new type of light. We will report results of the grower's decision.

Poster 33

Case Report: Determining the Etiology of Unusual Tumors in Chickens

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Two groups of 40- to 44-week-old live broiler breeder hens from one flock were submitted for determination of the etiology of visceral tumors. The flock had been vaccinated at hatch with a bivalent MDV vaccine (HVT + SB-1); mortality had increased to 1.5% per week, with a cumulative mortality of 12%. The most consistent lesion in necropsied chickens was extensive tumors involving the intestines; many birds had tumors in multiple organs. Microscopic examination of tumors revealed that the lesions were primarily lymphoblastic or myeloid in nature; lymphosarcomas, histiocytomas, and fibromas were also identified. Other tissues contained a mix of myelocytic and lymphocytic infiltrations. Additional diagnostic tests included virus isolation, PCR (for MDV, ALV-J, and reticuloendotheliosis virus), and immunohistological staining of fixed tissues.

Although ALV-J was not confirmed by virus isolation or PCR, the histologic appearance of tumors suggested that ALV-J was involved. Furthermore, the isolation of serotype 1 MDV from peripheral blood lymphocytes and its demonstration by PCR in tumor tissue suggest infection with MDV. Thus, the cause of tumors in this case appears to be the result of a combined infection with MDV and ALV-J. This case illustrates the fact that standard diagnostic methods do not always produce unequivocal diagnoses, especially in cases of multiple infections.

Poster 34

Lead Contamination of Eggs

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A group of 20 mixed breed chickens (heavy layers) were known to have consumed lead-containing paint chips. The levels of lead in blood, eggs (yolk, albumen, and shell), and tissues (liver, kidney, muscle, and ovary) from 6 of these chickens were determined.

The blood analysis indicated Pb levels ranged from less than 50 ppb to 760 ppb. Lead contamination of the yolks ranged from less than 20 ppb to 400 ppb and shells contained up to 450 ppb, but albumen contained no detectable amounts. There was a strong correlation between the amounts of Pb found in blood and egg yolks but no good correlation existed between blood lead levels and lead deposition in the shells. Of the tissues analyzed, kidney Pb levels were highest with livers ranking second. Muscle and ovary contained only small quantities of lead.

Lead contamination of egg yolks represents a potential public health hazard, especially to children consuming eggs from small, family-owned flocks.

Poster 35

Case Report: Vitamin A Deficiency in Bobwhite Quail

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Three laboratory cases will be presented in which gross and histologic lesions were consistent with vitamin A deficiency in bobwhite quail.

Poster 36

Modified PCR for Sexing Monomorphic Psittacines

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Pet bird industry personnel of Pacific Northwest requested Avian Health and Food Safety Laboratory of Washington State University to develop a PCR test to sex the monomorphic psittacines. Presently the sexing of psittacines in performed by an examination of chromosomes or using open surgery and visualizing the gonads. A PCR assay for avian sexing has been reported in the literature and modifications were made to improve sensitivity and separation of bands for rapid visualization. Evaluation of feather tips and blood were performed from previously sexed or from the mature breeders birds with positive results from all individuals for which DNA was extracted. Field samples have been processed with equal success.

Poster 37

Blindness in Broiler chicks due to Retinal Dysplasia

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Thirty-three nine-day-old chicks were examined over one-week period because of blindness. The incidence of blindness was 0.5% in a flock of 25,000 birds. Clinical examination revealed that these chicks lacked pupillary as well as fundus reflexes. Histopathology of the eyes revealed degeneration and rosette formation of photoreceptors. Since most of the chicks were from the same breeder source a genetic etiology is suspected.

Poster 38

Comparison of the Enzyme Linked Immunosorbent Assay for the Detection of Antibody to Newcastle Disease Between Commercial Flocks and Non-commercial Chickens Raised in the Backyard of Poor Families From Argentina

Celina Buscaglia

A commercial Newcastle Disease Virus (NDV) enzyme-linked immunosorbent assay (ELISA) was performed to 460 layers serum samples for NDV antibodies. These layers belong to poor people that raise the hens in their backyard. Since Argentina has been declared free of velogenic NDV with vaccination in 1998 and these birds can be a potential source of the virus, the purpose of this study was not only to check for the presence of antibodies, but to compare the values to sera from a commercial layer farm with a known vaccination program.

Poster 39

Development of an Inactivated Newcastle Disease Virosome Vaccine that Protects against Challenge from Velogenic Texas GB

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While outbreaks of highly virulent Newcastle disease virus (NDV) are a major concern to the poultry industry internationally, economic losses caused by low virulent (lentogenic) strains continue to result from decreased egg production in layers and airsacculitis in broilers. Since lentogenic strains of NDV are widely used as vaccine strains, it is possible that these viruses may be directly or indirectly responsible for some of the economic losses. In an effort to examine protection against NDV, we developed a non-replicating virosome ND (vNDV) vaccine that retains the ability to hemagglutinate chicken red blood cells and fuse with chicken embryo cells. Preliminary vaccine efficacy studies indicate that two intranasal doses containing 10 mg of vNDV protects chickens against lethal challenge from velogenic Texas GB.

Poster 40

Persistence of Avian Pneumovirus in Turkeys Post–Exposure and Length of Immunity

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Currently, there is no information in the literature to indicate the length of persistence of Avian Pneumovirus (APV) in infected birds. Two-week old turkey poults, negative for APV antibody, were divided equally into two isolation rooms. Birds in one group were inoculated intranasally with the 1999 Minnesota isolate of APV. After four days and at 3, 6, 9, 12, 15 weeks of age, five banded birds from a second group (APV unexposed) were transferred into the isolation room housing the APV exposed birds. At the end of 5, 10 and 14 days of commingling, the five banded birds were examined for the presence of APV by using RT-PCR and virus isolation. We collected blood samples from all birds at weekly intervals to examine seroconversion to APV. If there was any contact spread from the exposed birds or the environment (litter) to the APV unexposed birds, we would expect to see clinical signs and/or the presence of antibodies to APV. The results of this study with discussions on length of persistence of Avian Pneumovirus in turkeys post-exposure and degree of immunity will be presented.

Poster 41

Sequential Pathogenesis of Experimentally Produced Infectious Proventriculitis: Microscopic and Ultrastructural Changes

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Co-author: H. John Barnes

Infectious proventriculitis was experimentally reproduced in specific-pathogen-free chickens by intraocular inoculation of proventricular homogenates from proventriculitis-affected broiler chickens. Necrosis of alveolar secretory epithelium was evident in proventriculi beginning on day 3 postinoculation (PI); necrosis was not observed in proventricular mucosal epithelium or tubular epithelium. Mild inflammatory changes were detected beginning at 7 days PI in connective tissue stroma; however, inflammatory changes characteristic of the disease (infiltrates of lymphocytes, macrophages and plasma cells) were largely absent until day 10 PI. Concurrent infection with infectious bursal disease virus ameliorated damage of alveolar secretory epithelium and development of inflammatory responses. Ultrastructural studies are in progress.

Poster 42

Reo-Virus Infections in Broilers in Upper Egypt

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Summary
The widespread nature of Reo-virus infections among chicken flocks was proved by results of serological and virus isolation techniques. Reo virus was incriminated in cases showing arthritis (tenosynovitis), stunting and respiratory distress.

Serum samples taken from 54 broiler flocks of 4 –6 weeks of age and subjected to agar jell precipitation (AGP) and Elisa tests showed that 28 flocks were positive by both tests, while additional 6 flocks were positive only by AGP test.

Six strains were isolated from cases showing either tenosynovitis, malabsorption, or respiratory distress. Isolates were identified serologically.

Poster 43

Sequence Analysis of the S3 Gene from a Novel Turkey Reovirus

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The deduced o2 protein sequence from the S3 gene segment of a novel turkey reovirus isolated from the bursa of birds exhibiting poultry enteritis and mortality syndrome was determined. The NC98 S3 open reading frame comprised 1101 base pairs and encoded 366 amino acids with a molecular mass of 40.5 kDa. While the predicted o2 protein from chicken isolates share >95% identity, they share only 76-78% identity with NC98. Phylogenetic analysis of the deduced o2 amino acid sequence demonstrates that NC98 separates as a unique virus relative to other avian strains. The results of this study indicate that NC98 is a novel turkey reovirus that shares limited homology to isolates of chicken and duck origin.

Poster 44

Case Report: Reovirus Outbreak in Mississippi

Magee, Danny L.
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After experiencing loss of uniformity in broiler flocks, one production company noticed an increase in lameness and swollen gastrocnemius tendons. Investigation identified one breeder flock as the primary source of the affected broilers. Progeny of the sister flock did not appear to be involved. Review of the pullet flock history and serology suggested that the affected breeder flock had experienced a recent exposure to reovirus. The pullet vaccination program included reovirus vaccines and had been in place for many months. The problem prompted adjustments to the vaccination program for both broilers and breeders.

Poster 45

A Review of the Poultry Diseases of Diagnostic Findings in North Alabama

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This will summarize the significant diseases being seen in the densely populated North Alabama area and show the trends of ages, seasonality, immunosuppression – relatedness, etc. in broilers, breeders, commercial layers, and backyard birds as they are presented to the diagnostic lab system.

Poster 46

Resistance Pattern of Salmonella and e.Coli Strains Isolated from Poultry

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Summary
In order to establish a successful medication program on national basis, the antibiotic sensitivity pattern should be periodically studied. This study aimed to evaluate the susceptibility of Salmonella (66) and E. coli isolates (1130) recovered from affected chicken flocks during the year 2000.

Salmonella showed the highest sensitivity to quinolones and gentamycin. E.coli strains showed the highest susceptibility to quinolones, gentamycin, streptomycin and lincospectin and moderate sensitivity to chloramphenicol and amoxycillin.

Additionally, MIC values were determined for 30 strains of Salmonella and E. coli for Enrofloxacin. The values of MIC of Enrofloxacin sensitive Salmonella and E. coli strains ranged from 0.125 – 01.5 mcg/ml.

Poster 47

Cellular Immune Responses to *Samonella Enteritidis* Vaccination

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The effects of *Salmonella enteritidis* vaccination on the host cellular immune response was evaluated. Chickens were vaccinated subcutaneously with killed *S. enteritidis* vaccine at the ages of 3 and 5 weeks. After vaccination, serum samples were collected for 3 weeks for the evaluation of IFN-y and IL-2 levels. The IFN-y level was significantly ($p<0.05$) higher in the vaccine group at 7, 11, 14, and 21 days post vaccination (dpv) whereas the IL-2 level was higher at 14, 18, and 21 dpv. Therefore, killed *S. enteritidis* vaccine induce important chicken cytokines which are involved in the elimination of the intracellular Salmonella.

Poster 48

Effect of Formaldehyde Usage on In-Ovo Injected Eggs

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Two methods of administering formaldehyde, dose and constant rate infusion (CRI), were used in hatchers containing non in-ovo and in-ovo injected eggs. A non-disinfected hatcher received distilled water. At piping, the aerosol bacterial load in the hatchers receiving formaldehyde was significantly less than in the non-disinfected control hatcher. At hatch, the CRI was overwhelmed and only the dose hatcher had a significantly lower aerosol bacterial count. The CRI hatcher had significantly higher percent hatch of fertile compared to non in-ovo injected eggs exposed to water. In-ovo injected eggs in water and dose treatment groups contained significantly higher percentage of contamination than non in-ovo injected eggs. This difference has only numerical significance in the CRI treatment groups. Chicks from the CRI in-ovo injected eggs had a statistically significant decrease in feed conversion ratios when compared to water non in-ovo injected. All formaldehyde exposed chicks had numerically lower feed conversion as compared to the water exposed chicks.

Poster 49

Oxytetracycline Water Soluble Products: Comparison of Several Characteristics for Various Commercial Products

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Co-author: Bob Kempa

Oxytetracycline is a broad-spectrum antimicrobial approved for use in broilers (chickens), turkeys and swine. There are two pioneer NADAs for oxytetracycline hydrochloride water-soluble powder and over six branded medications on the market. Withdrawal times and specific label indications can vary depending on the manufacturer's label. Once a customer decides to judiciously use an oxytetracycline product, selection can be based on physical characteristics, in addition to price, label indications, withdrawal times, and tissue distribution. Physical characteristic parameters, including solubility and HPLC analysis, were evaluated for four branded products. Results will be discussed.

Poster 50

Microbiological Challenges of Commercial Turkey Flocks and Methods of Control

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Poultry production companies continue to strive to produce birds in the most economically efficient manner possible. Understanding how microbiological challenges impact performance and developing methods to reduce their effect is a prerequisite to improving the efficiency in poultry production systems. The objective of this study was to quantify the microbiological challenges facing turkeys through feed, water and litter and to evaluate current management practices to reduce these challenges. Feed, water, water-line and litter samples were collected from 14 turkey houses at seven production

facilities. Total bacteria, total gram-negative bacteria, coliforms, *E. coli* and *Staphylococcus aureus* populations were enumerated using standard procedures. Feed samples from all houses had total counts $<1 \times 10^5$ cfu/g, $<3.5 \times 10^3$ coliforms and $<1 \times 10^3$ *S. aureus* and *E. coli*. Water samples taken from lines leading to the plasson or nipple drinkers had very low bacterial levels. At all houses, total counts were $<1 \times 10^4$ cfu/g, and coliform and *E. coli* counts were $<1 \times 10^1$ cfu/ml. Chlorination and ozone treatment were effective at controlling bacterial levels in water. Water samples collected from catch trays on the nipple lines and plassons had much higher bacterial counts. The total counts ranges from 1×10^4 to $>1.0 \times 10^6$ cfu/ml while the coliforms ranged from $<1 \times 10^3$ to $>1.0 \times 10^6$ cfu/ml. Only one house had detectable levels of *E. coli* (7.0×10^3 cfu/ml) in the water collected from a catch tray. Analysis of swabs from water-lines indicated total counts ranged from 2.0×10^2 to $>1.0 \times 10^6$ cfu and only two sites had houses with coliforms $>1.0 \times 10^3$ cfu and confirmed *E. coli*. One of these sites had water and water-line swabs of *S. aureus* $>1.0 \times 10^3$ cfu/ml. No other samples had *S. aureus* $>1.0 \times 10^2$ cfu/ml. The frequency of cleaning plassons and nipples drinkers was correlated to reduced bacterial levels. Litter analysis indicated total gram-negative counts ranged from 1.0×10^5 to 3.6×10^9 cfu/g while coliforms ranged from 1.0×10^3 to 2.0×10^8 cfu/g. Litter *E. coli* populations ranged from 1.0×10^3 to 5.8×10^7 cfu/g. Management practices reducing litter water activity resulted in lower litter microbial populations.

Poster 51

Live Attenuated and Killed Salmonella Vaccines Cause an Increase in Cell-mediated Immunity among Laying Hens

Uma Babu

Co-authors: Mashassi Okamura, Dennis Gaines, Michael Myers, Richard Raybourne, Hyun Lillehoj and Robert Heckert

This study was conducted to investigate the differential impact of live and killed *Salmonella enteritidis* (SE) vaccines on cell-mediated immunity of 16 and 32 week old White Leghorn hens. The hens were vaccinated with the 2 vaccines and two weeks later CMI was assessed using splenic mononuclear cell proliferation, in response to Con A and antigen and cell subpopulation numbers as indices. Mitogen and SE flagella mediated proliferation and the cell populations were determined by ^{3}H-thymidine uptake and flow cytometry, respectively. Con-A mediated and the SE flagella mediated proliferation were enhanced in the 16 week old and 32 week old birds vaccinated with live vaccine, compared to the corresponding control birds. However, Con A-mediated response was higher in the killed vaccine group of only the 16-week old birds compared to the corresponding control birds. Furthermore, there was a significant increase in the SE-flagella mediated proliferation in the killed vaccine group compared to the control group in case of 32-week old birds. These functional changes were accompanied by some changes in the splenic mononuclear cell subpopulations, which included increased CD4 population in the live vaccine group compared to the control and the killed vaccine groups. Correspondingly, there was a reduction in the CD8 population in the live vaccine group, compared to the killed vaccine group. Overall, live vaccine appeared to result in greater functional changes, such as non-specific mitogen and antigen-specific proliferation of splenic lymphocytes compared to the killed vaccine. This may prove beneficial in protecting hens that are infected with the wild type SE.