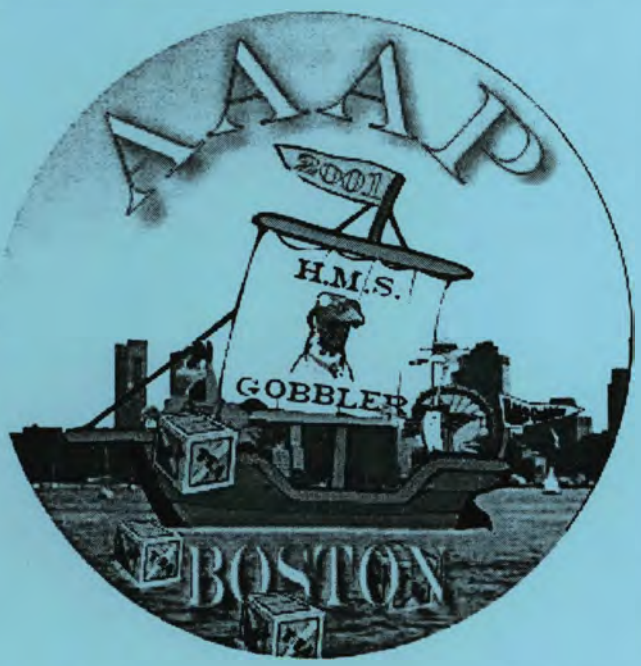
AAAP/AVMA Scientific Program



Boston, Massachusetts
July 2001

Respiratory Diseases of Poultry Symposium
Sunday, July 15, 2001
Convention Center Room 312

	Moderator: Sherrill Davison	
	Infectious Bronchitis Virus	
8:00 am	Diagnostic Tests for Infectious Bronchitis Virus – Advantages and Limitations of the Currently Used Techniques	Mark Jackwood
8:15 am	S1 Sequence Analysis; A Tool for Understanding Infectious Bronchitis Virus Outbreaks	Jack Gelb
8:30 am	Infectious Bronchitis in Pennsylvania	Sherrill Davison
8:45 am	The Current Status of the Detection and Typing of Infectious Bronchitis Virus	Fred Hoerr
9:00 am	**Discussion**	
	Infectious Laryngotracheitis	
9:15 am	Current Field Status of Infectious Laryngotracheitis	John Smith
9:30 am	Tracking Infectious Laryngotracheitis in the Field	Maricarmen Garcia
9:45 am	New Approaches in Infectious Laryngotracheitis Vaccine Development	Calvin Keeler
10:00 am	**Discussion**	
10:15 am	**Break**	
	Mycoplasma Gallisepticum	
10:45 am	*Mycoplasma gallisepticum* in North Carolina: 1999-2000	David Ley
11:00 am	Some Perspective on *Mycoplasma* Diagnosis and Control	Stanley Kleven
11:30 am	**Discussion**	
11:45 am	**LUNCH**	
	Moderator: Fred Hoerr	
	Avian Influenza	
1:30 pm	National and International Avian Influenza Outbreaks and Strategies for Control	David Swayne
	Newcastle Disease	
2:00 pm	Newcastle Disease Virus Pathotyping: The Current Emphasis on the ICPI	Jack King
2:15 pm	Molecular Evolution of Newcastle Disease Virus and the Application of Molecular Diagnostics	Bruce Seal
	Pneumoviral Infection	
2:30 pm	Epidemiology of Avian Pneumovirus and Host Range	K.V. Nagaraja
2:45 pm	Pathogenesis and Detection of Avian Pneumovirus Infection	Daniel Shaw
3:00 pm	**DISCUSSION**	
3:30 pm	**Adjourn**	

2001 AAAP/AVMA SCIENTIFIC PROGRAM
Boston, Massachusetts
Convention Center

	Monday, July 16, 2001 Session A Room 311	Monday, July 16, 2001 Session B Room 312
	Moderator: Carol Cardona	**Moderator:** Chuck Hofacre
8:00	**Molecular Characterization of Oncogenic Avian Leukosis Virus Subgroup J** Zavala, Guillermo, Mark Jackwood, & Deborah Hilt	**Rapid and specific detection of Campylobacter sp., Salmonella sp. and E. coli O157 with multiplex PCR-ELISA** Hong, Yang, Margie Lee, & John Maurer
8:15	**Classical and Molecular Analysis of Nested RT-PCR ALV-J Positive Plasma Samples undetected by Virus Isolation Coupled with Antigen Capture ELISA** El-Attrache, John, Pedro Villegas, & Maricarmen Garcia	**Frequency and location of *Salmonella enteritidis* contamination in eggs associated with various routes of experimental infection of laying hens** Gast, Richard, Jean Guard-Petter, & Peter Holt
8:30	**The Effect of immunosuppression caused by cyclophosphamide treatment of broiler chickens in Avian leukosis virus subgroup J (ALV-J) infection** Yongbaek, Kim, Tom Brown, Mary Pantin-Vera, & Saad Gharaibeh	**Use of new lateral flow panel for detecting Group D Salmonella, to rapidly determine *Salmonella enteritidis* (SE) presence in egg contents** Holt, Peter, Kun-Ho Seo, Richard Gast, & Henry Stone
8:45	**Natural history of Avian Leukosis Virus-Subgroup J in a closed flock of broiler breeder chickens over three years** Brown, Thomas, Nancy Stedman, Mary Pantin, Saad Gharaibeh, Kim Yongbaek, & Justin Verner	**Salmonellas from poultry products, environment and from the diagnostic samples** Dhillon, A. Singh, P. Roy, L. H. Lauerman, D.M. Schaberg, D. Bandli, & S. Johnson
9:00	**Evidence for myeloblastosis associated virus type-1 in a commercial White Leghorn stock** Spencer, J. Lloyd, Maria Chen, Susan Nadin-Davis, & Bernhard Benkel	**Broad serotype screening test for serologic diagnosis of Salmonella in poultry** Dirk Mekkes, Andrea Ballagi, Harold van den Heijden, & Valerie Leathers
9:15	**Effects of maternal antibodies against ALV-J on severity of ALV-J infection in broiler chickens exposed to hatch mates shedding virus** Gharaibeh, Saad, Tom Brown, Mary Pantin, & Kim Yongbaek	**Genetic characterization of antibiotic-resistant Salmonella from commercial poultry operations** Liljebjelke, Karen, Tongrei Liu, Charles Hofacre, & John Maurer
9:30	**Evaluation of experimental vaccines for subgroup J avian leukosis virus** Fadly, Aly, Lucy Lee, & Henry Hunt	**Interaction of *Salmonella typhimurium* and Infectious Bursal Disease virus (IBDV) in broiler chickens** Bautista, Daniel, Elankumaran Subbiah, Robert Heckert, Gretchen Oshop, Lenita Moura & Jane Wilson
	Break 9:45 AM – 10:15AM	**Break 9:45 AM – 10:15AM**
	Moderator: Richard Witter	**Moderator:** John Glisson
10:15	**Investigation of the Role of non-MHC alloantigen systems on Marek's disease pathogenicity in broilers** Wakenell, Patricia, W. Elwood Briles, Maung San Myint, Thomas Farver, & Donald Link	**Different control approaches of a highly virulent *Salmonella enteritidis* infection in chicken** Barbour, Elie, Gida Banat, Mohammed Farran, Obeid Faroon, & Faris Jirjis
10:30	**Characterization of Marek's Disease Virus gene encoding an IL8-like chemokine** Cui, Xiaoping, Lucy Lee, & Sanjay Reddy	**Construction and evaluation of a live attenuated vaccine to protect chickens against Group C Salmonella** Roland, Kenneth, Steve Gort, Steve Tinge, Beth Warner, & Mark Campbell
10:45	**Recombinant technology to mutate Marek's disease virus genes, pp38 is not essential for oncogenesis** Reddy, Sanjay, Blanca Lupiani, Robert Silva, Lucy Lee, & Richard Witter	**Comparison of fecal shedding of avian bacterial pathogens and parasites from free-living passerines and waterfowl** Morishita, Teresa, Dawn Fallacara, Clifton Monahan, Elizabeth Ley, Pyone Aye, & Brian Harr
11:00	**Virulence of Marek's disease virus: role of the 132 bp repeats** Silva, Robert, Blanca Lupiani, & Sanjay Reddy	**Potential human health consequences of use of antimicrobial agents in chickens** Kathryn Gay, Shannon Rossiter, & Jennifer Wright
11:15	**Insertional mutagenesis of a putative immunogenic epitope of pp38 of Rispens Strain of Marek's Disease virus into Md5** Lee, Lucy, Zhizhong Cui, Sanjay Reddy	**Effects of products from con. A stimulated peripheral blood lymphocytes on different bacteria** Don Reynolds, & Ali, Akbar,

	Monday, July 16, 2001 *cont'd.* Session A	Monday, July 16, 2001 *cont'd.* Session B
11:30	**Distinct pathogenesis of transient paralysis and persistent neurological disease revealed by differential attenuation of Marek's disease virus** Gimeno, Isabel, Richard Witter, Henry Hunt, Sanjay Reddy, & Ulrich Neumann	Open
	AAAP Awards Luncheon **11:45 AM – 2:00 PM**	**AAAP Awards Luncheon** **11:45 AM – 2:00 PM**
	Moderator: Thomas Brown	**Moderator:** Stanley Kleven
2:00	Open	Open
2:15	**Improved efficacy of partially attenuated vaccines for Marek's disease** Witter, Richard	**Association between hepatitis E-like virus and Hepatitis-Spenomegally Syndrome in Chickens** Shivaprasad, H.L., P. R. Woolcock, D.H. Read, J. Jeffrey, & X.J. Meng
2:30	**Epidemiology of an outbreak of Reticuloendotheliosis in breeder and market age turkeys** Crespo, Rocio, H.L. Shivaprasad, Cheryl Hall, & Aly Fadly	**The epidemiology of *Mycoplasma gallisepticum* in North Carolina. An update.** Martinez, Algis, Jean-Pierre Vaillanourt, David Ley, & Charlotte Smith
2:45	**Pathology of Reticuleondotheliosis in breeder and market age turkeys** Shivaprasad, H.L., Rocio Crespo, Cheryl Hall, & Aly Fadly	**Observations on the use of the random amplified polymorphic DNA analysis test on *Mycoplasma gallisepticum* cases in Colorado** Wooming, Brian, & David Ley
	Break **3:00 PM – 3:30PM**	**Break** **3:00 PM – 3:30PM**
	Moderator: David Swayne	**Moderator:** David Ley
3:30	**Pathobiology of a Hong-Kong origin H5N1 avian influenza virus in pigeons, geese, and emus** Perkins, Laura & David Swayne	**Pathogenicity of *Mycoplasma gallisepticum* field isolates for chickens and turkeys** Sanei, Babak, David Ley, & H. John Barnes
3:45	**The H5N2 Avian Influenza makes its debut in Central America** Senne, Dennis, David Suarez, Janice Pedersen, & Brundaban Panigrahy	**Detection of *Mycoplasma synoviae* in chicken tissues by in situ DNA hybridization with catalyzed signal amplification** Lockaby, Susan, Frederic Hoerr, & Lanqing Li
4:00	**Difficulties associated with the isolation and identification of avian influenza virus** Woolcock, Peter, Michael McFarland, & Steven Lai	**Comparison of the pathogenicity of recent isolates of *Mycoplasma synoviae* in turkeys** Kleven, Stanley, Min Su Kang, Victoria Leiting, & W.D. Hall
4:15	**Isolations of Non H5 or H7 Avian Influenza subtypes from backyard Pennsylvania poultry flocks** Henzler, David, Bruce Schmucker, & Philip DeBok	**Clearance of *Mycoplasma synoviae* from the upper respiratory tract by local treatment with antibody and tiamulin** Ramirez, Antonio, Eliana Icochea, Norma Noe, & Sharon Levishon
4:30	**Impact of H9N1 avian influenza infection in a layer farm** Lucio-Martinez, Benjamin	**Response to treatment of mycoplasmosis in broiler breeders based on evaluation by pipped embryo analysis** Payne, Albert, William Serna, & Tony Unandar
4:45	**Mortality Reduction in Leghorn Hens under a Modified Electromagnetic Field** Keirs, Robert	**Effect of Enrofloxacine and Tylosin on protection against MG challenge in birds vaccinated with TS-11** Contreras, Manuel, Rafael Fernandez, & Enrique Montiel
5:00	**Adjourn**	**Adjourn**

	Tuesday, July 17, 2001 Session A Room 311	Tuesday, July 17, 2001 Session B Room 312
	Moderator: Pedro Villegas	**Moderator:** Alex Bermudez
8:00	**Reliable virulence measures for the Newcastle disease virus (NDV)** Panigrahy, Brundaban, Dennis Senne, & Janice Pedersen	**Evaluation of Avian *E. coli* Iss Monoclonal Antibodies** Foley, Steven, Shelley Horne, Michael Robinson, & Lisa Nolan
8:15	**Virulence of six heterogenous-origin Newcastle disease virus isolates for domestic chickens** Kommers, Glaucia, Daniel King, Bruce Seal, & Corrie Brown	**Involvement of Iss in Avian *Escherichia coli* virulence and complement resistance** Horne, Shelley, Cathy Giddings, Richard Wooley, & Lisa Nolan
8:30	**Passive immunization protects birds following challenge with virulent NDV** Reynolds, Donald, & Sevinc Akinc	**The role of arsH in Avian *Escherichia coli*** Gibbs, Penelope, Richard Wooley, & John Maurer
8:45	**Newcastle disease vaccine failure in commercial broilers** Senne, Dennis, Bruce Seal, Daniel King, Eduardo Rivera, & Cesar Villarreal	**Apralan soluble powder for the control of colibacillosis-related mortality in growing chickens** Rings, Bret, Gregory Moore, & Alan Zimmerman
	Break **9:00 AM – 9:30 AM**	**Break** **9:00 AM – 9:30 AM**
	Moderator: Jagdev Sharma	**Moderator:** Dick Slemons
9:30	**Rapid detection and strain differentiation of infectious bursal disease virus in formalin-fixed, paraffin-embedded tissue** Pantin, Mary, Thomas Brown, Mark Jackwood, & Heather Ainsworth	**Characterization of recent *Pastuerella multocida* isolates from the West and Midwest** Angrick, Elisabeth, Pyone Aye, & Teresa Morishita
9:45	**Detection of single and multiple nucleotide polymorphisms in infectious bursal disease viruses using real-time RT/PCR** Jackwood, Daral, & Susan Sommer	**Evaluation of the effect of heating on oil emulsion *Pasteurella multocida* bacterin on reducing tissue reaction without affecting immunity** Burns, Karen, Jaime Ruiz, & John Glisson
10:00	**Pathogenicity of recent IBDV isolates inoculated in commercial broilers using in situ hybridization** Banda, Alejandro, Pedro Villegas, John El-Attrache, & Corrie Brown	**Lesions associated with an accidental head injection with a live fowl cholera vaccine** Gilbert, Robinette
10:15	**Involvement of cytokines in the pathogenesis of infectious bursal disease virus (IBDV) in chickens** Rautenschlein, Silke, Hung-Yueh Yeh, Moses Njenga, & Jagdev Sharma	**Extraction, preparation, and efficacy of *Pasteurella multocida* capsule and outer membrane proteins in Pekin ducks** Eltayeb, Amna, Jessie Price, & Mike Collins
	Business Meeting **10:30 AM – 12:00 Noon** **Lunch** **12:00 Noon – 1:00 PM**	**Business Meeting** **10:30 AM – 12:00 Noon** **Lunch** **12:00 Noon – 1:00 PM**
	Moderator: Mo Saif	**Moderator:** John Barnes
1:00	Open	Open
1:15	**Cytokines modulate IBDV vaccination** Yeh, Hung-Yueh, Silke Rautenschlein, & Jagdev Sharma	**Factors affecting pathogenesis of avian pneumovirus in turkeys** Jirjis, Faris, Sally Noll, David Halvorson, Kakambi Nagaaja, Alberto Back, & Daniel Shaw
1:30	**The role of T cells in protection by inactivated IBDV vaccine** Sharma, Jagdev, Silke Rautenschlein, & Hung-Yueh Yeh	**Neonatal avian pneomovirus infection in commercial turkeys** Halvorson, David, H.J. Shin, F.F. Jirjis, M.C. Kumar, M.K. Njenga, D.P. Shaw, S.L. Noll, & K.V. Nagaraja
1:45	**Infectious Bursal Disease Virus Proventriculitis** Kelly, Tami, Joe Giambrone, & Kalen Cookson	**Immune response of turkeys following intranasal vaccination with BPL-inactivated avian pneumovirus and live-virus challenge** Kapczynski, Darrell, & Cassandra Smith
2:00	**Comparison of monoclonals-based AC-Elisa, RT/PCR-RFLP and histology for the diagnosis of IBDV.** Lamichane, C.M.	**Effect of Fermentable Diets on Enterocyte Maturation and Turkey Coronavirus Infection** Pierson, William, Chanin Tirawattanawanich, & Calvert Larsen
2:15	**Pathogenesis of chicken infectious anemia virus: studies on latency** Miller, Myrna, & Karel Schat	**Effects of turkey coronavirus infection on turkey breeder hen performance** Rives, David, & James Guy

	Tuesday, July 17, 2001 *cont'd.* Session A	Tuesday, July 17, 2001 *cont'd.* Session B
	Break **2:30 PM – 3:00 PM**	**Break** **2:30 PM – 3:00 PM**
	Moderator: John Smith	**Moderator:** David Rives
3:00	**California 99 and Nebraska 95 strains of infectious bronchitis virus: Molecular and serological characterization** Jackwood, Mark, & Deborah Hilt	Open
3:15	**Virological and serological identification of IBV strains known as DE-072 variants** Villegas, Pedro, Miguel Ruano, John El-Attrache, & Naola Ferguson	**Production effects of exposure to Coban to turkey breeder hens in production that had previous exposure to Coban during rearing as breeder replacements** Trites, James, & Ronald Lippert
3:30	**The Pathogenesis and Genetic Characteristics of Nephropathogenic Infectious Bronchitis viruses isolated in the United States** Lee, Chang-Won, Deborah Hilt, & Mark Jackwood	**Pseudoaspergillosis in young turkey poults caused by Staphylococcus aureus** Barton, James
3:45	**Protection afforded by commercial vaccines against the Nebraska 95 IBV field isolate** Alvarado, Ivan, Pedro Villegas, John El-Attrache, & John Glisson	**Efficacy of Fenbendazole against round worms (A. dissinnilis) in commercial turkeys** Karunakaran, Daniel
4:00	**Prevalence of Infectious Bronchitis virus during the downtime period in broiler houses** Ruiz, Jaime, John Glisson, & Karen Burns	**Reduced 2-week mortality in turkey poults following administration of tylosin soluble** Evans, Robert, & Daniel Karanukaran
4:15	**Isolation and characterization of multiple strains of Infectious Bronchitis Virus (IBV) from a commercial layer farm** Lu, Huaguang, Q. Yang, T. Ward, P. Dunn, & D. Weinstock	**Turkey Enteritis – Gross and Histologic Evaluations from the field** Cummings, Timothy
4:30	**Management of an endemic Infectious Bronchitis 072 variant in a commercial broiler complex** Payne, Albert	**Characterization of a reovirus isolated from PEMS causing liver lesions** Schat, Karel, Priscilla O'Connell, Cherilyn Heggen-Peay, & Muquarrab Quereshi
4:45	**Field observations from the use of a vaccine reaction score system for IBV on broiler performance** Venne, Daniel	**The pathogenesis of turkey astrovirus infection in poults** Behling-Kelly, Erica, Stacey Schultz-Cherry, Matt Koci, Laura Kelley, & Corrie Brown
5:00	Adjourn	Adjourn
	Wednesday, July 18, 2001 **Session A** **Room 311**	**Wednesday, July 18, 2001** **Session B** **Room 312**
	Moderator: Willie Reed	**Moderator:** Buzz Klopp
8:00	**A recombinant fowl pox virus containing IBV-S1 gene and its potential for a vaccine** Khan, Mazhar, Xiuqing Wang, William Schnitzlein, & Deoki Tripathy	**The concurrence of *Staphylococcus aureus* with broiler ascites** Keirs, Robert, Chinling Wang, & Donnie Zumwalt
8:15	**Homologous fowlpox virus derived promoters for the development of recombinant vaccines** Srinivasan, Viswanathan, William Schnitzlein, & Deoki Tripathy	**Four-year summary of the disease incidence of broiler breeders in north Alabama** VanSambeck, Francene, Frederic Hoerr, Susan Lockaby, & Tami Kelley
8:30	**Efficacy and safety of a recombinant fowl pox virus containing Laryngotracheitis genes** Moore, Kristi, Jennifer Davis, Yoshinari Tsuzaki, David Hout, Motoyuki Esaki, Takahi Okuda, & Joan Leonard	**Production loss and mortality in broiler breeders from suspected salinomycin toxicity** Roney, Charles S., & Jeffery Courtney
8:45	**Analysis of *Mycoplasma gallisepticum* genes expressed by a fowl pox virus vector** Tsuzaki, Yoshinari, Jennifer Davis, David Hout, Shuji Saitoh, Motoyuki Esaki, Takashi Okuda, Ayumi Fujisawa, & Joan Leonard	**Antibiotic sensitivity profiles of *E. coli* isolates from commercial broiler chickens submitted to the Salisbury Animal Health Laboratory from 1998-2000** Tablante, Nathaniel, & Fidelis Hegngi
9:00	**Diseases and Lesions in the integument** Julian, Richard	**Rofenaid disk-diffusion assay results** Clark, Steven
9:15	**Role of management in control of fowl pox in laying chickens** Spasojevic, Radivoje	**Pathologic characterization of four Michigan Infectious Laryngotracheitis virus isolates** Fulton, Richard, Roger Maes, & Cunqin Han

	Wednesday, July 18, 2001 *cont'd.* Session A	Wednesday, July 18, 2001 cont'd. Session B
	Break **9:30 AM – 9:45 AM**	**Break** **9:30 AM – 9:45 AM**
	Moderator: Mick Fulton	**Moderator:** Ken Opengart
9:45	**Genetic and antigenic characterization of a poxvirus from ostrich** Tripathy, Deoki, Tae-Joong Kim, H. L. Shivaprasad & Peter Woolcock	**Haemoproteus lophortyx infection in bobwhite quail** Cardona, Carol, Bill Johnson, & Arthur Ihejirika
10:00	**Infectious coryza outbreak in a large egg layer complex.** Opitz, H.M.	**New, reliable, non-radioactive colorimetric assay to monitor lymphocyte proliferation during *Eimeria tenella* infection** Lillehoj, Hyun, Tadashi Miyamoto, & Wongi Min
10:15	**A caveat in FPV vaccines and their recombinants, a need for better vaccine** Singh, Pratik, William Schnitzlein, & Deoki Tripathy	**Coccidiosis control and immunity development using Clinacox (diclazuril) or Salinomycin in extended withdrawal programs** Mathis, Greg, & Marcelo Lang
10:30	**Immunogenicity of recombinant plasmid DNA expressing the VP2 capsid protein gene of infectious bursal disease virus and chicken interleukin-2** Hulse, Diane, & Carlos Romero	**Productive performance of broilers protected with a nonattenuated Eimeria vaccine** Icochea, Eliana, Miguel Adriano, Antonio Ramirez, & Pablo Reyna
10:45	**DNA vaccination with plasmids containing various fragments of large segment genome of infectious bursal disease virus** Wu, Ching Ching, Hua Chen Chang, & Tsang Long Lin	**The performance of Maxiban® (Narasin and Nicarbazin) and Nicarbazin in broiler starter rations with and without Bacitracin Methylene Disalicylate** Rings, Bret, & Robert Cochrane
11:00	**Determination of the most effective route for *in ovo* delivery of DNA vaccines** Moura, Lenita, Robert Heckert, Subbiah Elankumaran, & Gretchen Oshop	**An evaluation of the association between gut pH, feed passage, coccidial lesions and *Clostridium perfringens* in broiler chickens submitted to the Salisbury Animal Diagnostic Laboratory** Hegngi, Fidelis, Nathaniel Tablante, Pierre Brunet, & Pete Warnner
11:15	***In ovo* Nucleic Acid Immunization of the chicken against Infectious bursal disease and Newcastle disease** Oshop, Gretchen, Robert Heckert, Subbiah Elankumaran, & Lenita Maura	**The effects of chemical litter treatment on *Clostridia perfringens* induced necrotic enteritis in broiler chickens; a pilot study verifying a laboratory model** Martin, Michael, Patricia Wakenell, Randolph Chick, & Chris O'Brien
11:30	**Adjourn**	**Adjourn**

2001 AAAP/AVMA POSTER PROGRAM
Convention Center Room 310

1. **Relative importance of biosecurity measures: A Delphi Study**
   Vaillancourt, Jean-Pierre, & Algis Martinez
2. **Biological and molecular characterization of a novel quail herpes virus**
   Link, Donald, Karel Schat, & Patricia Wakenell
3. **The Effects of Riemerella (Pasteurella) anatipestifer on Pekin Ducks**
   Sarver, Craig, Teresa Morishita, Y.M. Saif, & Bedros Nersessian
4. **Experimental inoculation of pigeons (columba livia) with Mycobacterium bovis**
   Zwick, Laura, Scott Fitzgerald, & Willie Reed
5. **Avulsion of the Common Retinaculum in Meat Turkeys**
   Crespo, Rocio, Cheryl Hall, & Yan Ghazikhanian
6. **Antigenic relationship of turkey coronavirus isolates in the U.S.**
   Lin, Tsang Long, Chien Chang Loa, Ching Ching Wu, Thomas Bryan, Tom Hooper, & Donna Schrader
7. **Development and validation of a competitive enzyme-linked immunosorbent assay for detection of turkey coronavirus-specific antibodies**
   Guy, James, Jamie Breslin, & Lynda Smith
8. **Analysis of the polyprotein catalytic site on OH-infectious bursal disease virus (IBDV) strain protein VP4 by site-directed mutagenesis**
   Rodriguez, Juan, & Frederick Kibenge
9. **Trafficking in the chicken of recombinant plasmid DNA expressing the VP2 capsid protein gene of infectious bursal disease virus**
   Hulse, Diane, & Carlos Romero

10. **The sequence evidence of swine influenza viruses infecting chickens and turkeys**
    Suarez, David
11. **Genetic diversity of *Campylobacter jejuni* from broiler chickens at processing**
    Jeffrey, Joan, Karen Tonooka, Joan Lazano, & Allison Hunter
12. **Isolation and characterization of fluroquinolone-resistant Campylobacter sp. from poultry samples**
    Ghori, Hashim, & Mohammed Nawaz
13. **Effects of maternal antibodies on ALV-J infection in broiler chickens: parenteral injection of virus**
    Gharaibeh, Saad, & Tom Brown, Mary Pantin, and Kim Yongbaek.
14. **Efficacy of water administration of sodium acid sulfate in reducing crop contamination during feed withdrawal**
    Byrd, J. Allen, & Trisha Marsh Johnson
15. ***Salmonella enteritidis* cross contamination of table eggs**
    Boulianne, Martine, & Serge Messier
16. **Initial screen for presence of Salmonella in poultry environments with nested-PCR**
    Liu, Tongrui, Elizabeth Bartlett, Charles Hoface, Susan Sanchez, & John Maurer
17. **Genetic variability of reticuloendotheliosis provirus in the genome of fowlpox virus**
    Schnitzlein, William, Pratik Singh, & Deoki Tripathy
18. **Molecular characterization of Reticuloendotheliosis Virus (REV) insertions in the genome of field and vaccine strains of fowl pox virus (FPV)**
    Narang, Neelam, Maricarmen Garcia, William Reed, & Aly Fadley
19. **Condor Poxvirus with biological differences from Fowlpox Virus**
    Kim, Tae-Joong, & Deoki Tripathy
20. **Characterization of a possible avian macrophage chemoattractant from an avian macrophage EST library**
    Bliss, Travis, Marlene Emara, & Calvin Keeler
21. **Effect of toe trimming, probiotics and litter acidification on the incidence of cellulitis in broiler chickens**
    Tessier, Michelle, Philippe Labelle, Robert Gauthier, & Martine Boulianne
22. **Correlation of phenotypic and genotypic characteristics and embryo lethality in identifying virulent and commensal avian *Escherichia coli***
    Wooley, Richard, & Penelope Gibbs
23. **C3b deposition on avian *Escherichia coli***
    Nolan, Lisa, Catherine Giddings, Shelley Horne, & Richard Wooley
24. **Plasmid location of *iss* in an Avian *Escherichia coli* isolate**
    Johnson, Timothy, Catherine Giddings, Shelley Horne, & Lisa Nolan
25. **Screening a genomic library of a virulent avian *Escherichia coli* isolate for iss**
    Lynne, Aaron, Shelley Horne, Catherine Giddings, & Lisa Nolan
26. **Prevalence of Enteropathogenic *Escherichia coli* in naturally-occurring cases of poult enteritis-mortality syndrome**
    Pakpinyo, Somsak, H. John Barnes, David Ley, & James Guy
27. **Development of methods for detection of envelope glycoprotein of subgroup J Avian Leukosis Virus**
    Li, Maoxiang, Lucy Lee, & Aly Fadly
28. **Using ultrasound to predict lean tissue mass in broiler breeder hens**
    Dixson, Sallee, Robert Teeter, & Kenneth Powell
29. **Feline aminopeptidase N as a receptor for Infectious Bronchitis virus**
    Wang, Chinling, Betty Miguel, & Gregory Pharr
30. **Glycosylation analysis of the IBV spike glycoprotein**
    Wade, Emma, Mark Jackwood, & Deborah Hilt
31. **Synthesis and analysis of hammerhead ribozymes targeted to Infectious Bronchitis Virus subgenomic mRNA coding for the nucleocapsid protein**
    Callison, Scott, Mark Jackwood, & Deborah Hilt
32. **Use of a live coccidial oocyst vaccine to change a salinomycin-resistant field *Eimeria maxima* population**
    Newman, Linnea, Harry Danforth, Steve Roney, & John McCarty
33. **Immunogenetic Assessment of Staphylococcal Tenosynovitis and Osteomyelitis in broiler breeder stock**
    Bessinger, Kellye, Frederic Hoerr, Sandra Ewald, & Vicky Van Santen
34. ***Staphylococcus aureus* granulomatous pneumonia in 6-day-old turkey poults**
    Bermudez, Alex, & Magalie Boucher
35. **Correlation of tracheal antigenic distribution and levels of specific humoral antibodies in pneumovirus infected broiler breeders and their offsprings**
    Barbour, Elie, Faris Jirjis, & Shady Hamadeh
36. **Reproductive tract tumors in mature laying hens**
    Barnes, H. John, Gustavo Rodriguez, & Donna Carver
37. **Ontogeny of cellular and humoral immune response parameters ducks**
    Wang, Y., PR China, & U. Neumann
38. **Inestigations on immune cell populations of the head-associated lymphoid tissues: immunohistochemical aspects, cell mediated and humoral immune response in unvaccinated and vaccinated broilers**
    Dumrongsoontornchai, P., U. Neumann
39. **Drumstick lesions of unknown etiology at a poultry slaughtering /processing facility**
    Davis, Meredith, Benjamas Promsopone, & Teresa Morishita

Diagnostic Tests for Infectious Bronchitis Virus: Advantages and Limitations of Currently Used Techniques

Mark W. Jackwood
University of Georgia, Department of Avian Medicine, Poultry Diagnostic and Research Center

Infectious bronchitis virus (IBV) causes a highly contagious, acute upper respiratory tract disease in chickens. Different serotypes of IBV do not cross protect. Thus, diagnosis and serotype identification of IBV is critical to vaccine selection. Virus neutralization (VN) tests in embryonating chicken eggs are the gold standard and have been used to group IBV isolates into different serotypes but that test is laborious and expensive. An indirect ELISA test was developed using serotype specific monoclonal antibodies (Mabs). But, the Mabs for that test are only available for virus strains Ark99, Conn46, and Mass41. Two types of molecular based tests have been developed; the serotype specific reverse transcriptase-polymerase chain reaction (RT-PCR) test, and the RT-PCR/ restriction fragment length polymorphism (RFLP) method. The serotype specific RT-PCR method uses PCR primers specific for the Ark, Conn, Mass, DE072, JMK, and California serotypes of IBV. The RT-PCR/RFLP method utilizes a set of PCR primers designed to amplify the entire S1 gene of all IBV strains. That 1720 bp amplicon is then digested with restriction enzymes and the resulting pattern of bands can be used to identify all of the currently recognized serotypes of the virus, as well as variants of IBV. Sequencing the S1 glycoprotein gene can also be used for the identification of any IBV strain. Using sequence data, phylogenetic trees are generated which correlates with the serotype of the virus, and can be used to determine the similarity of that virus with other IBV serotypes.

S1 Sequence Analysis; Tool For Understanding IBV Outbreaks

Jack Gelb, Jr., Calvin L. Keeler, Brian S. Ladman, and Bruce F. Kingham
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Tools used to identify infectious bronchitis virus (IBV) field isolates have changed over the last decade. Today, isolates may be identified by analysis of the nucleotide sequence of the S1 spike glycoprotein gene that encodes many of the unique antigenic properties of the virus. S1 sequencing identifies new variants by their sequence variations. Alignment of S1 sequences of variant strains with those of existing strains provides a basis for comparison.

S1 sequencing has been used as a tool to study the epidemiology of outbreaks by following genetic changes over time as the viruses evolve, leading to a better understanding of the disease and possible control measures. The ongoing nephropathogenic outbreak in Pennsylvania will serve as an example.

Infectious Bronchitis in Pennsylvania

Sherrill Davison, Andre F. Ziegler, Jack Gelb, Jr., Patricia A. Dunn, and Robert J. Eckroade
New Bolton Center, University of Pennsylvannia (Davison and Eckroade)
Lasher Laboratory, University of Delaware (Ziegler)
Dept of Animal and Food Sciences, University of Delaware (Gelb)
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Infectious bronchitis is a viral disease of chickens that primarily causes respiratory disease but certain serotypes of the virus may produce kidney disease (nephropathogenic). Respiratory infections caused by infectious bronchitis virus are common in broilers and layers in the United States. In other countries, the nephropathogenic form of infectious bronchitis is also an important cause of disease and mortality in broilers and young pullets.

Since December 1997, 25 cases of infectious bronchitis involving a particularly nephropathogenic strain of virus have been confirmed in Pennsylvania. A wide variety of poultry have been affected by this particular virus strain, including eighteen broiler flocks, four commercial pullet flocks, two commercial leghorn layer flocks and one commercial layer breeder flock. It is unclear, at this time, how this virus was introduced and spread between flocks.

Compared with other affected groups, morbidity and mortality appeared greatest in diseased broiler flocks. Characteristic

gross lesions in broilers and pullets consisted of severe, diffuse renal swelling, with an increase in uric acid crystal retention in ureters and tubules. Gross necropsy findings in the affected layer and layer breeder flocks predominantly consisted of urolithiasis / visceral gout and moderate upper respiratory disease, respectively. The currently available modified live infectious bronchitis vaccines do not protect chickens against these nephropathogenic strains.

The Current Status of the Detection and Typing of Infectious Bronchitis Virus

Frederic J. Hoerr
C.S. Roberts Veterinary Diagnostic Laboratory

This is an invited presentation that will place perspective on traditional detection and serotyping methods for infectious bronchitis virus, with comparisons to the more recent RT-PCR technology, RFLP identification, and sequencing of the viral genome. Emphasis will be placed on the relative merits of the various tests and our ability, as an animal health discipline, to rapidly and accurately generate critical information for control of infectious bronchitis.

Current Field Status of Infectious Laryngotracheitis

John A. Smith
Fieldale Farms Corp.

A survey of diagnostic laboratories in the major poultry producing areas of the US will be conducted in the late spring of 2001. Data about the incidence, distribution, clinical features, and control measures used for Infectious Laryngotracheitis will be collected and reported as part of the AAAP Respiratory Disease Symposium.

Tracking Infectious Laryngotracheitis (ILT) in the Field

Maricarmen García
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Infectious Laryngotracheitis is an acute upper respiratory disease of poultry that spreads fast in the field. The disease is caused by alpha-herpesvirus Infectious Laryngotracheitis Virus (ILTV). To better control the disease all possible sources of outbreaks need to be identified. Epidemiological studies have demonstrated that vaccine-derived strains circulating in the field can be the source of outbreaks. The objective of this study is to determine where in the field these strains linger. In order to accomplish this objective a sensitive nested PCR assay specific for ILTV has been developed. Using this assay ILTV DNA has been detected in tracheal tissues from clinical cases of respiratory disease where ILT was not a suspect. Under histopathological examination these trachea tissues did not presented the characteristic ILT lesions, and virus was not isolated or detected by direct fluorescent antibodies, indicating that birds are carrying the virus in the absence of clinical signs. Collection of trachea, eye lid, lungs, and trigeminal ganglia from flocks where subclinical ILT is suspected are being collected for further PCR analysis. In addition environmental samples from layer and broiler house with previous history of ILT outbreaks will be tested with this assay to determine if the virus stays in the chicken house environment. The identification of virus sources in the chicken house environment will lead to better management and prevention of the disease.

New Approaches In ILTV Vaccine Development

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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. We will begin with a brief overview of the benefits and pitfalls associated with the use of the current generation of live attenuated ILTV vaccines. We will then discuss the status of recombinant ILTV vaccine development and new approaches to ILTV vaccination including genetic vaccination technologies.

Mycoplasma gallisepticum in North Carolina: 1999-2000

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In 1999-2000, North Carolina experienced unprecedented outbreaks of *Mycoplasma gallisepticum* (MG) in commercial poultry flocks. Our efforts focused on MG isolation, and strain identification; and epidemiology consisting of field investigations, a case control study, and reporting. Random amplification of polymorphic DNA (RAPD), a PCR-based method of DNA fingerprinting, was used for MG strain identification. We identified four different 'RAPD types' among field isolates, and found that their fingerprints were different than MG vaccine strains (F, ts11 and 6/85) and the 'House finch' strain. RAPD type A was isolated from a single backyard flock and multiple houses of a nearby turkey breeder farm. Type B was isolated from a broiler breeder farm, but before the infection was recognized progeny were moved to a site near other poultry farms in a neighboring county. Type B became widespread and was involved in major foci of infections in eastern and western North Carolina. Type E was isolated from a single broiler breeder farm in the northwestern part of the state. RAPD type F was identified in northeastern North Carolina and involved a cluster of farms consisting of a broiler breeder flock and multiple flocks of progeny. RAPD fingerprinting of MG field isolates enabled us to learn more about the epidemiology of the outbreaks and validated the utility of strain identification. Evidence suggests that inadequate monitoring of some broiler breeder flocks, the movements and interactions of people, and lack or lapses of biosecurity were major factors contributing to this epidemic of MG in North Carolina.

Some Perspectives on Mycoplasma Diagnosis and Control

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In spite of long-standing control programs such as the National Poultry Improvement Plan, outbreaks of *Mycoplasma gallisepticum*, *M. synoviae*, and *M. meleagridis* still occur. High numbers of poultry raised in concentrated areas and a diminished emphasis on biosecurity may be contributing significantly to this. Nevertheless, the traditional methods of routine monitoring by serology, biosecurity, and use of single age production units are important in limiting the spread of these infections. Control is more difficult when multi-age production units are used and when there are large numbers of birds of different types in the area.

M. gallisepticum is often controlled in multi-age commercial egg operations by vaccination. Milder live vaccines are relatively safe, but there have been documented instances of "escape" of vaccine strains which cause clinical problems in neighboring flocks. A live *M. synoviae* vaccine is available in some countries, but not in the U.S.

Modern biotechnology techniques such as polymerase chain reaction are valuable diagnostic supplements to traditional methods of serology and isolation and identification of the causative agent. New techniques such as random amplified polymorphic DNA (RAPD) and sequencing of targeted genes of the organism are proving to be very useful in identifying wild-type as well as vaccine isolates and will contribute significantly to our understanding of the epidemiology of these infections.

National and International Avian Influenza Outbreaks and Strategies for Control

David E. Swayne
USDA, Agricultural Research Service, Southeast Poultry Research Laboratory

Reports of highly pathogenic avian influenza (HPAI) outbreaks are compiled and listed on the Office International des Epizooties (OIE) website, http://www.oie.int/, and in the bimonthly OIE Bulletin. However, the reporting of outbreaks of list A or B diseases is voluntary and some disease outbreaks that are common knowledge among the scientific community and media have not been reported to OIE. For example, the Hong Kong H5N1 HPAI outbreak in poultry was not reported to OIE. Other diseases such as mildly pathogenic avian influenza (MPAI) are not on list A or B and thus are not reported to OIE. This report compiles information from OIE, recent scientific literature and credible personal sources on avian influenza in the world. Much of the information is fragmentary and incomplete, but this report is the best as can be confirmed.

MPAI and HPAI (H7N1) in Italy - An outbreak of MPAI appeared in turkeys of northern Italy on 26 March, 1999. The AI virus was identified as H7N1 and was mildly pathogenic for chickens in laboratory tests and had a hemagglutinin cleavage site that lacked multiple basic amino acids consistent with other MPAI viruses. The MPAI virus spread and infected at least 199 farms by mid-December, 1999 - most in the area between Brescia and Verona - and included six farms of turkey breeders, 11 farms of broiler breeders, 12 farms of layers, 164 farms of meat turkeys, four farms of broilers, and two farms of guinea fowl. In turkeys breeders, 5-20% mortality, 30-80% drop in egg production, respiratory signs, inappetence, 'egg peritonitis' and misshapen and fragile eggs were reported. Broiler breeders and layers had respiratory signs and peritoneal lesions similar to those in turkey breeders. However, mortality rates and egg production drops were less than in turkey breeders. In meat turkeys, mortality varied from 5-97% depending on age and the presence of secondary pathogens such as *Riemerella anatipestifer*, *Ornithobacterium rhinotracheale*, *Mycoplasma* sp., paramyxovirus type 2, Newcastle disease virus, avian pneumovirus and adenovirus. Clinically, very young turkey poults had severe respiratory distress with gasping for air in the most severely affected birds. Gross lesions seen included fibrin clots in the trachea and sinuses, ruptured air sacs, and subcutaneous emphysema. In many cases, death occurred by suffocation. Other young poults developed severe necrotizing pancreatitis with accompanying diarrhea.

The strategy for control was serological monitoring, controlled slaughter and limiting shipments of eggs from infected breeders. The number of cases declined in the summer, but began to climb in the fall, 1999.

On 17 December, 1999, the outbreak took an abrupt change in character with poults exhibiting 100% mortality, nervous signs and widespread hemorrhages. The MPAI virus had a change in the hemagglutinin protein cleavage site from -PEIPKGR*GLF- to a HPAI virus with cleavage site of -PEIPKGSRVRR*GLF-. This appears to be an insert of four additional amino acids, two being basic amino acids, at the hemagglutinin cleavage site of the MPAI virus to make it a HPAI virus.

The last outbreak of HPAI was identified on 5 April 2000 in meat turkeys. In total, 13,732,912 birds were involved in 413 flocks and an additional 3-4 million were depopulated as a pre-emptive action. Birds affected included 8,118,929 layers; 2,692,917 meat turkeys; 1,625,628 broilers; 743,319 broiler breeders; 260,340 quail, ducks and pheasants; 247,379 guinea fowl, 42,276 turkey breeders, 387 ostrich and 1,737 backyard poultry. Most outbreaks occurred in Northcentral Italy in the regions of Lombardia (234) and Veneto (158), but isolated outbreaks were reported in Piemonte (6), Fruili (5), Emilia-Romagna (5), Sicila (2), Trentino (1), Sardegna (1) and Umbria (1).

MPAI (H5N2) in Mexico - A difficult issue in many underdeveloped countries is accurate, rapid diagnosis of avian influenza and differentiation from velogenic viscerotropic Newcastle disease (vvND). This is especially true because mortality patterns and clinical findings in the field can be similar between vvND and MPAI co-infected with secondary pathogens. Some confusion exists in Central America as to definitive diagnosis of field cases as vvND or AI. This has lead to false reports in media and internet sources, which have resulted in border closures to poultry movement between countries.

MPAI in United States Live-Bird Markets - Surveillance for AI viruses in various poultry species of the Live-Bird Markets (LBM) by the Departments of Agriculture in New York and New Jersey continues with the assistance of National Veterinary Services Laboratory (NVSL), Ames, Iowa. The report of isolations has been previously made by Dennis Senne in this proceedings and David Suarez reports later on molecular changes in these viruses.

MPAI (H9N2) in Asia - MPAI viruses (H9N2) have been reported to cause morbidity and mortality in countries across Asia, primarily in countries of the Middle East, and in Pakistan. In many cases, the clinical signs and mortality have been associated with secondary pathogens. Infections with H9N2 AI viruses without mortality have been reported in China and Hong Kong. Sequence data of the H9 and N2 gene of H9N2 AI viruses from Saudi Arabia, Iran, Pakistan and Hong Kong by Veterinary Laboratories Agency (Dennis Alexander, United Kingdom) and SEPRL, respectively, have shown they are all closely related and of the same virus lineage. H9N2 AI viruses were first reported in China in the mid-1990's and spread to the Middle East and Pakistan in the late 1990's. In the Middle East and China, the recent appearance of vvND has complicated the diagnosis and control of MPAI viruses.

HPAI (H5N1) in Hong Kong - During 2000, in Hong Kong, the Department of Agriculture, Fisheries and Conservation isolated several AI viruses (H5N1) from geese or swabs from goose cages in the wholesale market. In 1999, HPAI (H5N1) were isolated from the environment of LBM where geese and ducks were housed. Based on studies at SEPRL, the 1999 H5N1 viruses have the same hemagglutinin gene as the 1997 H5N1 AI viruses, but the other genes were from separate lineages.

These 1999 H5N1 viruses were similar to those circulating in domestic geese in south China in 1996. These 1999 H5N1 viruses were from birds imported from Mainland China and were highly pathogenic for chickens in experimental studies. Iran has reported avian influenza (H9N2) causing severe problems especially in areas where poultry are raised in close geographic locations and in the presence of unhygienic conditions. The outbreak began in 1997 and is ongoing. Cost estimates for 1998 alone were $11 million for 20 million meat chickens affected. Several breeder operations were also involved and these flocks had to be depopulated.

Over the past 3 years, avian influenza viruses (H9N2) have been isolated from chickens in Saudi Arabia. Mortality and morbidity has varied, but it is high when accompanied by secondary pathogens such as vvND or *Mycoplasma gallisepticum*. Avian influenza has not been reported in Syria, Jordan or Lebanon.

During 2000, there was widespread serological evidence of H9N2 infection of chickens in the LBM of Hong Kong, but no associated disease. MPAI viruses of H9N2 subtype were isolated from swabs collected from birds in retail markets.

Other AI issues EU definition - Currently, federal regulatory action is undertaken with HPAI and not MPAI. HPAI is defined as those viruses that kill 6, 7 or 8 of 8 inoculated susceptible chickens, or H5 and H7 AI viruses having a cleavage site with multiple basic amino acids as reported for previous HPAI viruses, or AI viruses that produce cytopathic effect in cell culture without exogenous trypsin. However, the EU is considering a change in definition to include all H5 and H7 AI viruses along with HPAI H5 and H7 AI viruses as requiring regulatory action. This is in response to the outbreak in Italy during 1999 and 2000 when a MPAI (H7N1) mutated and became a HPAI. They are also proposing the option of using vaccines with future outbreaks of H5 and H7 AI. Below is a summarization of the European Union Scientific Committee Recommendations as reported in the specific website http://europa.eu.int/comm/food/fs/sc/scah/out45_en.pdf:

1. "Avian influenza" means an infection of birds caused by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype. However, in making this recommendation the Committee was concerned at the current lack of knowledge on the prevalence of LPAI viruses of H5 and H7 subtypes in poultry populations. It would seem a wise precaution that before the recommendation is implemented serological surveys of poultry populations in Member States should be undertaken to determine this prevalence and the likely economic impact that would be involved

2. Throughout the EU there is a marked lack of surveillance for avian influenza, particularly in free-living birds, and yet routine surveillance could give an early warning of the prevalence of viruses of H5 or H7 subtype in the locality of domestic birds. Member States should put in place routine surveillance systems for the detection of influenza viruses in free-living birds.

3. Vaccination against influenza A viruses of H5 and H7 subtype should not normally be allowed. The possible use of emergency vaccination... should be retained. ....., it considers that there is a potential for a greater role of vaccination in the control of avian influenza, that could be realised by the development of novel marker vaccines.

4. In order to improve the efficacy of emergency vaccination as an aid to avian influenza control the Commission is urged to support the development of novel marker vaccines.

5. "Poultry" are all birds that are reared or kept in captivity for: the production of meat or eggs for consumption, the production of other commercial products, for restocking supplies of game or for breeding these four categories of birds.

6. The Committee, recognizing that there is at present no adequate in vitro alternative, agreed to the continued inclusion of an in vivo test for virus virulence ..., but with some reluctance. The Commission is urged to encourage and support further research into the development of in vitro tests aimed at replacing the use of birds in virulence tests for avian influenza.

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Newcastle Disease Virus Pathotyping: The Current emphasis on the ICPI

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Chicken inoculation has been utilized since the 1950s to differentiate virulence among isolates of avian paramyxovirus-serotype 1 (APMV-1), a term synonymous with Newcastle disease virus (NDV). Three tests, the intracerebral pathogenicity index (ICPI) in day-old chicks, intravenous pathogenicity index (IVPI) in 6-wk-old chickens, and intracloacal inoculation of 6- to 8-wk-old chickens, have been employed to make that differentiation across the full spectrum of NDV virulence which ranges from asymptomatic infections to high mortality in susceptible chickens. Results of the ICPI provide a differentiation of viruses of low virulence, the lentogenic vaccines, from viruses of moderate (mesogenic) or high virulence (velogenic). The IVPI and intracloacal inoculation provides a further differentiation of NDV mesogens from velogens and differentiation of the viscerotropic and neurotropic clinical forms of the more virulent viruses, respectively. Because there is an international trend to separate APMV-1 isolates into two virulence groups, the lentogens and a more virulent group comprised of previous mesogenic and velogenic viruses, the role of the ICPI as a differential test has become emphasized. This also expands the definition of NDV isolates that present a risk to poultry beyond the previous velogenic classification. During 1999, that new emphasis was codified when member countries of the Office International des Epizooties (OIE) approved a new definition of ND. ND is now defined by OIE as an infection with an avian paramyxovirus-1 (APMV-1) with virulence measured by the intracerebral pathogenicity index (ICPI) of 0.7 or greater or by a molecular criterion that is a virulence marker for those viruses, the amino acid sequence in the fusion protein cleavage site.

Molecular Evolution of Newcastle Disease Virus and the Application of Molecular Diagnostics

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Highly virulent Newcastle disease virus (NDV) isolates are List A pathogens, and it is compulsory that reports of its isolation be made to the Office of International Epizootes (OIE). The principle molecular determinant for NDV virulence is the fusion protein cleavage site amino acid sequence. The OIE now requires amino acid sequence of the fusion protein cleavage site along with biological assessments for NDV pathotype reporting. Degenerate oligonucleotide primers are used to reliably amplify nucleotide sequences that encode the fusion protein cleavage activation site utilizing NDV genomic RNA as a template. From viral genomic sequence data collected, evolution and virulence determinants of NDV isolates have been monitored.

Epidemiology of Avian Pneumovirus and Host Range

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Avian pneumovirus (APV) has been show to cause respiratory disease in turkeys and has been associated with swollen head syndrome in chickens. There appears to be no evidence on the susceptibility of species other turkeys and chickens to avian pneumovirus. In Minnesota Avian pneumovirus as a cause of respiratory disease in turkeys has resulted in heavy economic losses to turkey producers for the past 3 years. In an attempt to understand the bioecology of this virus, we have examined the susceptibility of ducks, broiler chickens, mice and rats to APV of turkey origin. The results of this study in the context of epidemiology of this virus will be presented.

Pathogenesis and Detection of Avian Pneumovirus Infection

Daniel Shaw
Penn State University

Three-week-old turkeys experimentally inoculated with a MN isolate of avian pneumovirus developed clinical signs within 2 days. The poults completely recovered by 10 days after inoculation. The clinical signs closely resembled those seen in the field and consisted of snicking, coughing, ocular and nasal discharge, and swelling of the sinuses. The postmortem changes consisted of sinusitis and rhinitis. There was very mild inflammation in the tracheas. Antibody against APV was detected by 6 days postinoculation using ELISA. Virus was consistently detected in the nasal turbinates on days 4 through 6 PI using the PCR test and immunohistochemical staining. Virus was not as reliably detected by PCR in tracheal swabs during this period. The virus was isolated from the nasal turbinates during the same time period.

Molecular Characterization of Oncogenic Avian Leukosis Virus Subgroup J

Guillermo Zavala, Mark Jackwood, and Deborah Hilt
The University of Georgia

The E element and the 3'LTR of forty-five ALV-J field isolates were sequenced after PCR amplification of proviral DNA from tumors or from infected fibroblasts. An intact or nearly intact E element was more frequently detected in tumor proviral DNA than in isolates from clinically healthy birds. Sequence data for the E element and the 3'LTR amplified from proviral DNA of tumors experimentally reproduced with one of the oncogenic ALV-J isolates were identical in all tumors isolated from various experimentally infected birds. The U3, R, and U5 regions of the LTR appeared highly conserved among most of the ALF-J isolates examined.

Classical and Molecular Analysis of nested RT-PCR ALV-J positive plasma samples undetected by virus isolation coupled with antigen capture ELISA

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Individual plasma samples were tested directly by nested RT-PCR. ALV-J positive samples were propagated on DF-1 cells and submitted for ALV detection by the p27 antigen capture ELISA test (ac-ELISA). Samples that failed detection by virus isolation coupled with ac-ELISA were analyzed by additional cell culture propagation along with PCR analysis. The presence or absence of ALV-J antibody and p27 gene sequence analysis was evaluated.

The Effect of Immunosuppression Caused by Cyclophosphamide Treatment of Broiler Chickens in Avian Leukosis Virus Subgroup J (ALV-J) Infection

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Avian leukosis virus subgroup J (ALV-J) infection causes significant economic losses due to mortality and decreased productivity in broilers. This study was performed to determine the effect of immunosuppression on ALV-J infection. 4 mg of cyclophosphamide was injected intraabdominally to broilers each day for 4 days from the first day after hatch. Birds were then infected with an isolate of ALV-J (ADOL-7501) at 2 weeks of age. Immunosuppression was confirmed by analysis of relative bursa weights and analysis of splenocytes using a flowcytometry and a mitogenesis assay. For 8 weeks post-infection, body weight gain, viremic status by diagnostic RT-PCR and quantitative competitive RT-PCR, and antibody status by virus neutralization test were measured. Comparisons will determine the effects of ALV-J infection and immunosuppression on these parameters.

Natural History of Avian Leukosis Virus-Subgroup J in a Closed Flock of Broiler Breeder Chickens Over Three Years

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One flock of commercial broiler breeder hens naturally infected with Avian Leukosis Virus-Subgroup J (ALV-J) was identified. 120 hens and 50 roosters were transported to the Department, and maintained in isolation from other poultry, vaccines, and infectious agents. ALV-J was periodically confirmed in each hen using a combination of ALV antigen capture ELISA, virus isolation on C/E cells, and RT-PCR. The egg production, fertility, rate of viral shed, hen/rooster mortality, tumor incidence and type, stability of viremia and antibody status, and molecular characteristics of the ALV-J present in this closed breeder flock over its three-year life were determined.

Evidence for Myeloblastosis Associated Virus Type-1 in a Commercial White Leghorn Stock

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A polymerase chain reaction (PCR) test that detected Rous associated virus-1 (RAV-1) failed to detect 3 field isolates of subgroup A avian leukosis virus. However, the test did detect a virus in an imported commercial stock of White Leghorns. Tests on whole blood from the commercial chickens and cultures that had been inoculated with blood yielded both 250 and 375 bp products (sizes estimated). Based on sequencing and phylogenetic analysis, the virus detected was considered to be myeloblastosis-associated virus type 1 (MAV-1).

Effects of Maternal Antibodies on ALV-J Infection in Broiler Chickens: Exposed to Hatchmates Shedding Virus

Saad M. Gharaibeh, Tom Brown, Mary Pantin, and Yongbaek Kim

This experiment was designed to determine the effects of in ovo antibody on horizontal virus exposure. Three groups of ALV-J negative embryonated chicken eggs were used. At 4 or 6 days of incubation, eggs were inoculated in the yolk sac as follows. Group 1 was inoculated with neutralizing antiserum against cloned ALV-J (ADOL-7501). Group 2 was inoculated with nonimmune serum. Group 3 was inoculated with cloned ALV-J (ADOL-7501) to serve as shedders. Embryos from the 3 groups were hatched and raised together until 13 weeks of age. Body weights, tumor formation, viremia status, and antibody status differences between groups will be discussed.

Evaluation of Experimental Vaccines for Subgroup J Avian Leukosis Virus

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Merial Inc., Gainesville, GA (Gaudry, Bublot)

Three experimental vaccines (two recombinant vaccines and a genetically-engineered cell line) expressing the envelope glycoprotein (gp85) of strain ADOL-Hc1 of subgroup J avian leukosis virus (ALV-J) were evaluated using commercial meat-type chickens or experimental line 0 white leghorn chickens. Using immunofluoresence, all three vaccines tested positive for expressing ALV-J gp85 in vitro; however, these vaccines failed to induce detectable neutralizing antibodies to ALV-J in vaccinated chickens. In one experiment, the incidence of ALV-J-induced viremia in vaccinated commercial chickens was lower than that in unvaccinated chickens, but only within 4-5 weeks following challenge with virus. Data suggest that one of the recombinant vaccines tested in this study may have a transient effect on the development of ALV-J-induced viremia.

Investigation of the Role of Non-MHC Alloantigen Systems on Marek's Disease Pathogenicity in Chickens

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The role of the major histocompatibility complex (MHC) on Marek's Disease pathogenicity has been well documented. However, the influence of other alloantigen systems on Marek's Disease susceptibility, with the exception of the Y system, has not been examined. We have been evaluating the possible influence of non-MHC alloantigen systems (A, C, D, E, H, I, L and P) on gross and microscopic tumor development after challenge with virulent Marek's Disease virus (RB1B) in inbred lines of chickens. Preliminary findings show that the P system does affect the pathogenicity of Marek's Disease virus.

Characterization of Marek's Disease Virus Gene Encoding an Interleukin-8 (IL8) -like Chemokine

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USDA, ARS, Avian Disease and Oncology Laboratory (Cui, Lee, Reddy)
Michigan State University (Reed)

Marek's disease virus (MDV), an alpha herpesvirus which induces cancer-like disease in chickens, is considered as a major disease problem in poultry industry. Recently, a new IL8-like gene encoded by MDV (vIL8) has been identified and its function in MDV is not known. Since MDV infection in vivo begins with cytolytic infection of B cells, followed by latent infection in T-cells. Therefore, the recruitment of B or T cells is crucial in the initial establishment of infection, and vIL8 may function in recruiting proper target cells for infection. We have identified vIL8 transcripts in MDV infected cells by Northern blot analysis. Using rabbit anti-vIL8 peptide serum, we localized vIL8 expression in the cytoplasm of MDV infected cells. The biological function of vIL8 using proteins expressed in baculovirus will be discussed.

Recombinant Technology to Mutate Marek's Disease Virus Genes: pp38 is not Essential for Oncogenesis

Sanjay M. Reddy, Blanca Lupiani, Robert F. Silva, Lucy F. Lee, and Richard L. Witter
USDA, ARS, Avian Disease and Oncology Laboratory

Five overlapping cosmid clones were constructed that contain the entire genome of a very virulent strain of Marek's disease virus (Md5). Transfection of chicken embryonic fibroblasts (CEF) with the five cosmids resulted in production of infectious MDV. Using RecA assisted restriction endonuclease technology we have deleted the pp38 gene from one of the cosmids. Transfection of CEF cells with the mutant cosmid, along with the other four cosmids, resulted in MDV that does not express the pp38 protein. The *in vitro* growth of pp38 deleted virus was similar to that of the parental Md5, however its growth was slightly impaired *in vivo*. Birds inoculated with pp38 deleted MDV showed that it is not essential for oncogenesis. This technology would be useful for studying the function of viral genes in MDV replication and establishment of latency.

Virulence of Marek's Disease Virus: Role of the 132 BP Repeats

R.F. Silva, B. Lupiani, and S.M. Reddy
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Oncogenic Marek's disease virus (MDV) can be attenuated by repeatedly passing the virus in cell culture. Concomitant with attenuation is the increase in the number of copies of a 132 bp region located in TRL and IRL of the viral genome. We have mapped the RNA transcripts that originate in and around the 132 bp repeat region and will show how these transcripts are altered as MDV is attenuated. We have also deleted one open reading frame in this region and will discuss how this deletion in a vvMDV has affected pathogenesis.

Insertional Mutagenesis of a Putative Immunogenic Epitope of pp38 of Rispens Strain of Marek's Disease Virus into Md5

Lucy F. Lee, Zhizong Cui, Barry Coulson and Sanjay Reddy
USDA, ARS, Avian Disease and Oncology Laboratory

Marek's disease virus (MDV) phosphorylated protein pp38 is one of the unique MDV proteins abundantly expressed in serotype 1 MDV and tumor cells. Substitution of one amino acid in Rispens pp38 resulted in reduced immune responses in chickens upon inoculation with the mutant virus. Using three monoclonal antibodies for pp38, we have identified a third epitope related to immunogenicity of pp38 of Rispens strain. Using overlapping cosmid technology we have switched the pp38 gene of Md5 with that of Rispens. Monoclonal antibody specific for Rispen pp38 was used to detect the expression of pp38 gene of Rispens in Md5.

Distinct Pathogenesis of Transient Paralysis and Persistent Neurological Disease Revealed by Differential Attenuation of Marek's Disease Virus

Isabel M. Gimeno
USDA, ARS, Avian Disease and Oncology Laboratory

Objective - Two neurological syndromes have been associated with highly virulent Marek's disease virus (MDV): transient paralysis (TP) and persistent neurological disease (PND). The aim of this study was to elucidate if TP and PND represent single or multiple biological processes as well as to relate pathogenesis of the infection in the CNS with other parameters of MDV infection. We also endeavored to validate the use of differential attenuation for discriminating among MDV biological functions.

Design and procedure - Three week old 15x7 chickens lacking maternal antibodies against the three serotypes of Marek's disease virus were used. In a first experiment chickens were inoculated with ten different passage levels of strain 648A (from 10 to 100). Clinical signs were checked daily through 84 days post inoculation and gross lesions evaluated at necropsy. In a second experiment chickens were inoculated with five passages levels of strain 648A (from 10 to 50) and samples of blood, brain, lymphoid organs and skin were periodically collected for virus isolation, viral DNA assay by PCR and viral antigen detection by immunohistochemistry.

Results - TP and PND were attenuated at different passage levels. While strain 648A lost the ability to induce TP between passage level 30 and 40 and appeared closely linked to parameters of viral replication, the ability to induce PND was lost between passage level 80 and 90, coincident with the loss of the ability to induce neoplastic lesions in peripheral nerves and other visceral organs.

Conclusions - TP and PND are unrelated pathologic syndromes and seem to be differently regulated. Also, the use of viruses at different passage levels with varying degrees of attenuation is presented as a useful tool for studying the pathogenesis of MDV infection.

Improved Efficacy of Partially Attenuated Vaccines for Marek's Disease

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

Objective - Serotype 1 Marek's disease (MD) viruses may be attenuated for oncogenicity by up to 100 serial passages (p100) in cell culture. This process occurs gradually. A fully attenuated virus no longer induces gross lymphomas in susceptible chickens while earlier passages retain various levels of oncogenicity. Studies were conducted to better understand the relationship between attenuation and induction of protective immunity by serially passaged serotype 1 viruses.

Design and animals - 15x7 chickens with maternal antibodies to all 3 serotypes of MD virus were immunized at hatching with viruses passaged until fully attenuated or at selected passages prior to full attenuation. Vaccinated chickens were challenged at 5 days post vaccination with virulent MD virus. Lymphoma frequency was determined through 8 weeks post challenge.

Results - In tests with 4 different virus isolates, the partially attenuated viruses consistently induced higher levels of protection than fully attenuated viruses; the mean advantage was 41% compared to fully attenuated counterparts. In a second trial, a partially attenuated, mildly oncogenic (p80) preparation of strain 648A provided 83% protection compared to 49% by the fully attenuated (p100) preparation. Significant protection also was provided by overtly oncogenic preparations of 648A (p40-60).

Conclusions - Partially attenuated, mildly oncogenic strains may induce strong protective immunity against virulent MD virus challenge. Even viruses with significant virulence are capable of inducing protective immune responses in some chickens. Partially attenuated viruses, although not currently permitted as commercial vaccines, may represent a unique strategy to induce strong protective immunity against MD.

Epidemiology of an Outbreak of Reticuloendotheliosis in Breeder and Market Age Turkeys

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University of California, Davis (Crespo and Shivaprasad),
Zacky Farms (Hall), USDA-ARS (Fadly)

Objective - Epidemiology of the transmission of reticuloendotheliosis virus (REV) between breeder flocks, their progeny, and market age turkeys.

Design - Descriptive report.

Animals - Female turkey breeders, between 13 and 54 weeks of age. Progeny flocks between hatch and market age. Unrelated affected meat turkey flocks, at market age.

Procedure - Health and vaccination histories of the flocks were reviewed. Necropsy, histologic evaluation, serologic tests, and virus isolations were performed.

Results - Increased mortality, lymphoma and decreased egg production was observed in a turkey breeding flock for over 20 weeks. REV was confirmed by virus isolation and serology. Progeny of these breeders was seropositive for REV at hatch, but the performance of these flocks was normal and neoplasia was not observed. One month after the breeding flock was diagnosed with REV a few meat turkey flocks from the same integrator experienced increased condemnation due to lymphoma in a few organs. The pox vaccine used in the breeders was negative for REV and meat birds were not vaccinated for pox.

Conclusions - The origin of REV in these breeders could not been determined. The previous diagnosis of REV in California was made over 15 years ago. REV outbreaks have been associated with contaminated pox vaccine; however the vaccine used in these breeders was negative for REV and meat birds were not vaccinated for pox.

Pathology of Reticuloendotheliosis in Breeder and Market Age Turkeys

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California Animal Health & Food Safety Laboratory System, Fresno Branch,
University of California, Davis (Shivaprasad and Crespo),
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Objective - To study the pathology of a natural outbreak of reticuloendotheliosis virus (REV) infection in breeder and market age turkeys.

Design - Descriptive case report.

Animals - Fifty breeder and market age turkeys between the ages of 13 and 54 weeks.

Procedure - Records on necropsy, histopathology and clinical pathology findings were reviewed. REV infection was confirmed by virus isolation and PCR assays.

Results - Thirty birds had involvement of the liver characterized by either pale diffuse moderate to severe enlargement or with multiple pale white nodules. Most of the 30 birds had severe diffuse pale and enlarged spleens, and a few birds had pale enlarged kidneys, thymus, cecal tonsils, mucosa of the intestine, conjunctiva, etc. Microscopically there was infiltration of neoplastic lymphocytes in the organs described above and in addition bone marrow, adrenal, ovary, thyroid and rarely bursa of Fabricius were also infiltrated with neoplastic lymphocytes. Blood chemistry analysis from 14 birds revealed increased levels of aspartate aminotransferase, creatine kinase, lactate dehyrogenase and alkaline phosphatase in most birds. One bird had severe leukopenia.

Conclusions - Gross and microscopic examination of various organs confirmed lymphoma in various organs such as liver, spleen, kidney, thymus, cecal tonsils, conjunctival lymphoid tissue, bone marrow, intestine and other organs.

Pathology of a Hong Kong Origin H5N1 Avian Influenza Virus in Pigeons, Geese, and Emus

Laura E.L. Perkins
USDA, ARS, Southeast Poultry Research Laboratory

Objective - To compare the susceptibility of pigeons, geese, and emus to intranasal (IN) inoculation with a H5N1 highly pathogenic avian influenza virus (HPAI).

Design - Two to seven birds of each species were inoculated with virus and 2-4 birds served as sham controls. Birds were evaluated for clinical disease, gross and histopathological lesions, and distribution of viral antigen. Virus reisolation was attempted from swabs and selected tissues.

Animals - Four 2-week-old emus (*Dramaius novaehollandiae*), 11 two-week-old Embden geese (*Anser anser*), and 10 four-week-old pigeons (*Columbia livia*) were used.

Procedures - Birds were intranasally inoculated with $10^{6.0}$ EID_{50} of the A/chicken/Hong Kong/220/97 (H5N1) HPAI virus (HK/220). All birds were monitored for clinical disease. Selected birds were euthanatized and sampled 2-14 days later for influenza virus and histologic lesions.

Results - Despite a lack of mortality in geese and emus species, both species developed neurological disease. The HK/220 virus was mostly neuro- and pancreatotropic, causing necrosis and inflammation which corresponded to the presence of viral antigen. Virus reisolation was most consistent from the brain. In contrast, pigeons lacked clinical signs and gross and histological lesions; viral antigen was not demonstrated in tissues; nor was virus reisolated from swabs or from tissues.

Conclusions - These results imply that emus and geese are susceptible to infection and disease following IN inoculation with the HK/220 virus. Conversely, pigeons are more resistant and likely played a minimal role in the epidemiology of the H5N1 Hong Kong-like HPAI viruses.

The H5N2 Avian Influenza Makes Its Debut in Central America

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USDA, Southeast Poultry Research Laboratory (Suarez)

In the year 2000, an outbreak of avian influenza (AI) occurred for the first time in Guatemala, a central American country bordering Mexico. The outbreak was caused by low pathogenic H5N2 virus. Both commercial poultry and backyard flocks were affected, and the infection was widespread in adjoining area of the capital, Guatemala City. Phylogenetic analysis of the virus showed that it was most closely related to the H5N2 virus isolated in Chiapas, Mexico in 1997, providing evidence that the H5N2 virus that has plagued Mexico since 1994 has spread to poultry in a neighboring country. An overview of surveillance and control programs as well as properties of the virus will be presented.

Difficulties Associated with the Isolation and Identification of Avian Influenza Virus

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University of California, Davis, Fresno Branch

Objective - To review methods used to isolate avian influenza virus (AIV) from clinical specimens.

Sample population - Clinical specimens submitted to the California Animal Health and Food Safety Laboratory System for virus isolation.

Results - Since 1993, 14 cases of avian influenza (AI) have been diagnosed by virus isolation from 8 avian species. In three of the cases, primary isolation attempts for AIV were negative when standard protocols for the isolation of AIV were followed. In each of these cases AIV could not be detected by hemagglutinating activity (HA), agar gel immunodiffusion (AGID) test or negative stain electron microscopy following serial passage in embryonating chicken eggs (ECE) via the

chorioallantoic sac route. However, primary isolations of AIV were achieved by inoculation into either the yolk sac or onto the chorioallantoic membrane of ECEs.

Conclusions - Our results emphasize the value of not relying on just one route of inoculation of ECEs for the isolation of AIV, and the advantages of using more than one test to identify and confirm AIV. A combination of HA and AGID tests and negative stain electron microscopy were used in all these cases.

Isolations of Non-H5 or H7 Avian Influenza Subtypes from Backyard Pennsylvania Poultry Flocks

David J. Henzler, Phil C. DeBok, and Bruce Schmucker
Pennsylvania Department of Agriculture

Eighteen flocks had isolations or serologic evidence of non-H5 or H7 avian influenza subtypes from backyard Pennsylvania poultry farms for the years 1999 and 2000. Waterfowl were present on fifteen farms concurrently with chickens, turkeys, and guinea fowl on thirteen, four, and three premises, respectively. Flocks were geographically distributed over eleven counties with six flocks in Lancaster County. Isolations occurred during ten of twelve months with March having three and September having five flocks. Flock size varied from ten to three thousand birds. Virus subtypes isolated from cloacal swabs included: H6N1, H6N4, H10N7, H4N6, H11N9, H2N3, and H11N9, predominantly from waterfowl. Pennsylvania tests approximately 119,000 blood samples, numerous diagnostic swabs, and auction market bird cloacal swabs for avian influenza annually.

Impact of H9N1 Avian Influenza Infection in a Layer Farm

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Avian influenza virus (AIV) type A, subtype H9N1 and *Pasteurella gallinarum* were isolated from sentinel chickens placed among a layer flock (Flock A) suffering from mild respiratory distress. Nasal exudate and sneezing had been observed in flock A. Egg production had dropped from 81 to 72% during a period of 35 days, and mortality increased from 0.2 to 0.3% per week.

Once AIV was identified (two months after disease outset) sentinel chickens were placed among flocks in the rest of the farm. Also, tracheal and cloacal swabs, and blood samples were obtained from the rest of the flocks in this multiple-age farm.

AIV antibodies were not detected in the affected flock, or in the rest of the farm. Low titers (1:8 to 1:64) of hemagglutination-inhibition (HI) antibodies against H9 were detected in birds from Flock A, but not in the rest of the farm.

AIV was not isolated from tracheal and cloacal swab samples. In the months following the AIV isolation, sentinel chickens placed in the farm have yielded only avian infectious bronchitis virus.

AIVs of low virulence may be more prevalent than expected, and detected only very sensitive methods of isolation, and antibody detection.

The H9N1 isolated in this case seems to be of so low pathogenicity that it did not establish itself even in a multiple-age farm.

Mortality Reduction in Leghorn Hens under a Modified Electromagnetic Field (EMFM)

Robert Keirs
College of Veterinary Medicine, Mississippi State University

EMFM were utilized in two producing flocks of the same breed and line. Trial I, comparison made to Breeder Goals (BG) and two previous flocks 2 and 4, the same house and feed. Trial II, concurrent comparison to similar house size, two wks. younger. Trial I to 60-wks (EMFM) mortality avgd. 0.052%/wk. (BG) 0.08%. Previous flocks 2 and 4, 0.072% and 0.08%. Trial I, cycle two thru 79-wks. (EMFM) avgd. 0.06%, (BG) 0.13%, flocks 2 and 4, 0.134% and 0.10%. Trial II cycle one (EMFM) avgd. 0.077%/wk and 0.087% nontreatment, cycle two 0.11% and 0.180%. Molting mortality to 50% production return. Trial I 65 to 73 wks. (EMFM) was 0.89%, (BG) 2.93%, 107 to 115 wks. 1.73% vs. 2.93%. Trial II molt of 8 wks. (EMFM) 0.62% vs. nontreatment 1.13%.

Monday, July 16, 2001
Session B

Rapid and Specific Detection of *Campylobacter sp.*, *Salmonella sp.*, and *E. coli* O157 with multiplex PCR-ELISA

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A multiplex PCR-ELISA method was developed to identify *Campylobacter sp.*, *Salmonella sp.*, and *E. coli* O157 in a single multiplex PCR reaction. *Salmonella invA*, *Campylobacter flhB*, and *E. coli* O157 *rfbB* genes were analyzed to design multiplex PCR primers. PCR products of unique sizes were detected. In the next step, biotin labeled oligo probes were designed to hybridize with the PCR products in the ELISA assay. Twenty-one strains of *Campylobacter*, 20 strains of *Salmonella* and 9 strains of *E. coli* O157 were included in the study. Multiplex PCR-ELISA proved to be a fast and sensitive method to identify those common food borne pathogens.

Frequency and Location of *Salmonella enteritidis* Contamination in Eggs Associated with Various Routes of Experimental Infection of Laying Hens

Richard K. Gast, Jean Guard-Petter, and Peter S. Holt
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Effective application of risk reduction practices such as refrigeration requires accurate information about the characteristics of *Salmonella enteritidis* contamination of eggs. Experimental infection models can be useful tools for understanding how *S. enteritidis* is deposited in eggs and for testing potential control strategies. Oral inoculation is presumed to provide the closest simulation of naturally occurring infections, but intravenous and aerosol inoculations have sometimes been associated with higher incidences of egg contamination. Determining the differences in the numbers and location of *S. enteritidis* deposited in eggs after inoculation by the various routes is necessary for understanding the relevance of the various experimental models. The present study compared the frequency and location of *S. enteritidis* deposition in egg contents following experimental inoculation by three different routes. In two replicate trials, specific-pathogen-free laying hens were infected with an *S. enteritidis* culture mixture prepared to optimize invasive behavior. Groups of hens received either an oral dose of 10^9 *S. enteritidis*, an aerosol dose of 10^9 *S. enteritidis*, or an intravenous dose of 10^7 *S. enteritidis*. Fecal shedding of *S. enteritidis* and serum antibody responses to *S. enteritidis* flagellin were monitored to characterize the experimental infections induced by the various routes of inoculation. Eggs laid during the first 4 weeks post-inoculation were cultured to detect and enumerate *S. enteritidis* in the yolk and albumen. The comparative patterns of deposition of *S. enteritidis* in eggs provided an indication of the potential usefulness of the different routes of inoculation as models for studying the relationships between egg contamination, bacterial multiplication, and control practices.

Use of a New Lateral Flow Panel for Detecting Group D *Salmonella* to Rapidly Determine *Salmonella enteritidis* (SE) Presence in Egg Contents

Kun-Ho Seo, Peter S. Holt, Richard K. Gast, and Henry D. Stone
Southeast Poultry Research Laboratory

We have been collaborating with Neogen Corporation, Lansing, Michigan, to develop an antibody-based lateral flow assay system to rapidly detect Group D salmonellae from poultry samples. As few Group D salmonellae infect poultry besides *Salmonella enteritidis* (SE), the test is fairly specific for SE. The need for a rapid and simple method for the detection of SE from egg contents, as well as other poultry samples, becomes more of an issue as the prevalence of on farm testing increases. The test panel, Reveal® for *Salmonella enteritidis* (SE), was easy to use, requiring 0.1 ml of sample administered into the sample port onto a nitrocellulose matrix, and which was read by observing lines formed by the reaction of organism with antibodies impregnated in the matrix. A study was conducted to examine the detection capability of the panels in fecal and egg samples. We found that 1) prior enrichment of sample was necessary to increase numbers of SE to detectable levels; 2) because of the viscosity of the egg samples, certain dilution techniques or extraction procedures were necessary in order to allow proper wicking of sample down the membrane. Dilution of egg contents or fecal samples, 1:10 in tetrathionate broth prior to 48 hr incubation increased detection of SE by the panels; 3) following incubation, autoclaving a portion of the enriched sample and then applying the supernatant to the panel dramatically increased the sharpness of the panel bands and subsequently the sensitivity and specificity of the test. The panel exhibited excellent specificity and good sensitivity and, because of the ease of use and interpretation, allows individuals with less microbiological sophistication to conduct the testing.

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Salmonellas from Poultry Products, Environment and from the Diagnostic Samples

A. Singh Dhillon, P. Roy, L.H. Lauerman, D.M. Schaberg, D. Bandli, and S. Johnson
Washington State University

Sero-groups B, C, D and E of the salmonellas were isolated from different sources of poultry. A trial was conducted in SPF chicks to compare the pathogenicity of 14 different salmonellas. *Salmonella pullorum* was used as control. Chicks were inoculated with 1′10 7 CFU of salmonellas by crop gavage at one day of age. Measurement of body weights, re-isolation of salmonellas from different organs, gross pathology and hispothology were considered to compare the pathogenicity. Reduced body weights were observed in many treatment groups. Ceca was 100% positive for salmonella re-isolation at both acute and chronic infection. Consistent pathology and histopathological changes include pericarditis, hepatitis, yolk sac infection and enteritis.

Broad Serotype Screening Test for Serologic Diagnosis of Salmonella in Poultry

Dirk Mekkes, Andrea Ballagi, Harold van den Heijden, and Valerie Leathers

Monitoring and controlling Salmonella infection in poultry seems recently to have gone through a renaissance because of the growing awareness and concern from the consumer side. The demand for large-scale screening is increasing which calls for simple and reliable commercial diagnostics. IDEXX Labs developed a new generation of Salmonella screening ELISA together with The Animal Health Services, Deventer, The Netherlands. The test kit detects a wide range of serotypes, it is user-friendly and suitable for even automated high though-put testing.

Genetic Characterization of Antibiotic-resistant *Salmonella* from Commercial Poultry Operations

K.A. Liljebjelke, C. Hofacre, T. Liu, and J.J. Maurer

The use of antibiotics in agriculture has been implicated as contributing to the emergence of drug resistance in food-borne human pathogens. Integrons are mobile genetic elements, involved in the dissemination of antibiotic resistance in gram-negative bacteria. This genetic element acquires drug resistance genes through the recombination of these genes into a specific integration site. The integrase gene, *intI*1 a signature of class 1 integrons, mediates this recombination event. This study uses PCR, southern blotting, and pulsed-field gel electrophoresis (PFGE) to "fingerprint" *Salmonella* isolated from two commercial poultry farms, and characterize their drug resistance genes in order to follow antibiotic resistance from the hatchery to the finished product. *Salmonella* isolates from chicken feces and litter were screened for class 1 integrons by southern blot using a DNA probe for *intI*1. Approximately 45% of isolates contain class I integrons. For *Salmonella* positive for *intI*1, the gene cassette present in class 1 integron was further characterized by PCR. Primers to 5' and 3' conserved sequences of the class I integron yield amplicons varying in size between 750 to 4,000bp. The antibiotic resistance genes present in the integron gene cassette were identified by PCR-ELISA. The majority of gene cassettes identified with PCR-ELISA contained aminoglycoside resistance genes. When this study is complete, analysis of the data will provide insight into the ecology of drug resistance in commercial poultry operations.

Interaction of *Salmonella typhimurium* and Infectious Bursal Disease virus (IBDV) in Broiler Chickens

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University of Maryland, College Park

Salmonellosis in poultry is of high public health importance because of its implication to human food-borne illnesses. The immunobiology of *Salmonella* in chickens in the face of concurrent infection with an immunosuppressive virus (Infectious Bursal Disease Virus) was investigated. *Salmonella*-IBDV interaction was determined by evaluating environmental shedding, cecal carriage, persistence, humoral response, and organ invasion of *Salmonella* through microbial quantification, immunohistochemistry and ELISA. Dually infected broilers had greater amounts of *Salmonella* in the ceca and environmental fecal samples than birds infected solely with *Salmonella*. Likewise, consistent gross lesions including fibrinous pericarditis, perihepatitis, peritonitis, ascites, and bursal atrophy were in seen only in this group. There was persistently high *Salmonella* shedding in all birds from both groups up to the end of the study (6 weeks post-infection). ELISA and immunohistochemistry results will be discussed at this presentation.

Different Control Approaches of a Highly Virulent *Salmonella enteritidis* Infection in Chicken

Elie Barbour, G. Banat, M. Farran, O. Faroon, and F. Jirjis

Control of infectivity by a highly virulent *Salmonella Enteritidis* (SE) in chicken was attempted by different approaches. The use of either SE bacterin immunopotentiated by thymulin and zinc, of live strains of attenuated SE, of competitive exclusion microflora, or of natural herbal immunopotentiators were evaluated based on immunity and protection against controlled challenges.

Construction and Evaluation of a Live Attenuated Vaccine to Protect Chickens Against Group C *Salmonella*

Kenneth L. Roland
Megan Health, Inc.

Objective - The purpose of this work was to develop a live attenuated vaccine strain to protect chickens against colonization by group C Salmonella.

Design, Animals and Procedure - We constructed two candidate vaccines: a *Dcya Dcrp* derivative and a *DphoP* derivative of *Salmonella hadar*. White Leghorn chickens were vaccinated at day of age and at two weeks with one of the two strains. A non-vaccinated group served as a control. At four weeks of age, all birds were challenged with wild-type *S. hadar* and necropsied 6 days later. Numbers of *S. hadar* in the ceca were determined.

Results - Both strains induced a serum antibody response against *S. hadar* LPS as determined by ELISA. The average OD for birds vaccinated with the *DphoP* or *Dcya Dcrp* derivatives was 0.456 and 0.881, respectively. Although the *Dcya Dcrp* derivative induced higher levels of serum antibody, the birds were not protected. Conversely, birds vaccinated with the *DphoP* strain showed significant protection against *S. hadar* challenge. Seventy percent of the non-vaccinates, 60% of the *Dcya Dcrp* vaccinates and 15% of *DphoP* vaccinates were positive for *S. hadar*. In a second experiment, birds were vaccinated with the *DphoP* strain only. After challenge, all of the birds in both groups were colonized. The geometric mean of cecal *S. hadar* isolated from the control group was 1.0 x 10^6 CFU/g and from the vaccinated group was 32 CFU/g, indicating a 4-5 log reduction in colonization by the challenge strain.

Comparison of Fecal Shedding of Avian Bacterial Pathogens and Parasites from Free-living Passerines and Waterfowl

Teresa Y. Morishita, Pyone Pyone Aye, Dawn M. Fallacara, Clifton M. Monahan, Elisabeth C. Ley, Brian S. Harr, and Raymund F. Wack
Ohio State University

There has been an increased concern regarding the role of free-living avian species in the transmission of diseases to the commercial livestock and poultry industry as well as to humans. Passerines and waterfowl are the primary species that are of concern and can serve as a reservoir for pathogens. The objective of this study was to survey these avian species for the presence of common bacterial pathogens (*Salmonella sp., Escherichia coli, Campylobacter jejuni,* and *Pasteurella multocida*) and endoparasites. The study design was to collect fresh fecal samples from free-living passerines and waterfowl from various locations in the State of Ohio. The procedure employed fecal culturing for the targeted bacteria species using standard microbiological techniques. To survey for endoparasites, a modified sugar flotation technique was utilized. Results demonstrated the presence of bacteria that could serve as a source of infection for livestock and poultry as well as for humans. In conclusion, free-living species should be monitored around livestock and poultry facilities as well as areas for human habitation to reduce the spread of diseases.

Potential Human Health Consequences of Use of Antimicrobial Agents in Chickens

Kathryn Gay, S. Rossiter, and J. Wright

Antimicrobial agents are commonly used in chickens to treat diseases, and in the absence of disease, for disease prevention and growth promotion. Such use creates selective pressure which can result in antimicrobial resistance which may be transmitted to humans through the food supply, and may compromise the treatment of infectious diseases in humans. We will review national surveillance data on enrofloxacin, gentamicin, ceftiofur, virginiamycin and bacitracin resistance in Salmonella, Campylobacter, and enterococci isolated from chickens purchased from grocery stores and humans which are apparently associated with the use of these antimicrobial agents in chickens, and discuss the potential human health consequences.

Effects of Products from con. A Stimulated Peripheral Blood Lymphocytes on Different Bacteria

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The objective of present study was to determine the effects of products secreted by the Con. A stimulated peripheral blood lymphocytes on the survivability of different bacteria. The effect was evaluated in vivo as well as in vitro. The results on the effects of these products on the growth of different bacteria will be presented.

Association Between Hepatitis E-Like Virus and Hepatitis-Splenomegally Syndrome in Chickens

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Hepatitis-Splenomgaly (HS) syndrome is a disease of both layer and broiler type chickens. The syndrome is characterized by decreased egg production and increased mortality with an onset around 30 weeks of age. The cause of this syndrome is not known. A virus was detected from the bile of some of the chickens with HS syndrome which has characteristics of Hepatitis E virus. Data on clinical signs, pathology, virus detection and molecular characteristics of the virus will be presented. Comparison of HS syndrome with Big Liver and Spleen disease of chickens described in Australia will be made.

The Epidemiology of *Mycoplasma gallisepticum* in North Carolina: An Update

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The objective was to describe a *Mycoplasma gallisepticum* (MG) epidemic and to identify associated risk factors. A case-control study was nested in a prospective investigation of an MG epidemic that occurred in North Carolina between October 1999 and February 2001. One hundred thirty farms were quarantined. Of these, 28 confirmed MG positive farms were visited as cases and 19 MG negative farms in proximity to cases were selected as controls. Serology results were available for all farms; case-control farms were visited and surveyed with a standardized questionnaire. Univariate analyses (Fisher Exact test) on data collected on these 47 farms suggest that relationships among farm workers and biosecurity issues were at the core of this epidemic. The risk of becoming MG positive appeared to be higher in areas of high farm density. Although MG positive backyard flocks have been directly associated with a few outbreaks, the presence of backyard flocks known to commercial growers in the vicinity of their farm was the same for MG positive and MG negative farms. No requirements for coveralls or boots for visitors was associated with the risk of becoming MG positive. Although the investigation is still ongoing, current evidence suggests that inadequate MG monitoring (interval between testing and delays in obtaining results) in 1999 and people movement coupled with the lack of biosecurity on several farms have greatly contributed to this epidemic.

Observations on the Use of the Random Amplified Polymorphic DNA Analysis Test on *Mycoplasma gallisepticum* Cases in Colorado

Brian Wooming and David Ley
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Pathogenicity of *Mycoplasma gallisepticum* Field Isolates for Chickens and Turkeys

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North Carolina State University, College of Veterinary Medicine

The North Carolina poultry industry experienced an outbreak of *Mycoplasma gallisepticum* (MG) from October 1999 to November 2000, involving more than 100 flocks. Random amplification of polymorphic DNA (RAPD), a PCR-based method of DNA fingerprinting, was used for Mg strain identification. We identified four different 'RAPD types' among field isolates. Of these isolates approximately 91% were type B, 4% type A, 4% type F and 2% type E. Questions concerning the pathogenic potential of the various RAPD types for poultry were paramount among commercial producers. The purpose of this study was to assess the pathogenicity of North Carolina MG field isolates, by experimental challenge in commercial-type chickens and turkeys and to evaluate the performance of commonly used diagnostic tests. Meat type chickens and turkeys were allocated into three inoculation groups (RAPD type B, S6 reference strain positive control, and sham inoculated negative control) of ten birds each. At 14 days of age, birds were challenged by combined conjunctival, intranasal and tracheal route. Birds were observed daily for clinical signs for up to 3 weeks post-inoculation. At seven-day intervals post inoculation, birds were sampled for serology (SPA, HI and ELISA), culture and PCR to assess the performance of these diagnostic tests. All birds were necropsied and visible lesions in nasal sinuses, tracheal mucosa and air sacs were scored on a scale of 0 to 3. Nasal sinus and tracheal tissues were also evaluated microscopically and tracheal mucosal thickness was measured.

Detection of *Mycoplasma synoviae* in Chicken Tissues by *in situ* DNA Hybridization with Catalyzed Signal Amplification

Susan B. Lockaby
C.S. Roberts Veterinary Diagnostic Lab

Objective - A nonradioactive *in situ* hybridization technique using catalyzed signal amplification was evaluated for detection of *Mycoplasma synoviae* DNA in formalin-fixed, paraffin-embedded chicken tissues.

Design/Procedure - A 207-bp DNA probe generated by polymerase chain reaction was purified, biotinylated, and used in a commercial *in situ* hybridization kit. The technique was applied to histologic sections of nasal passages, trachea, viscera, and tendons from *M. synoviae*-inoculated specific-pathogen-free broilers.

Results/Conclusions - Results will be presented and compared with conventional methods for diagnosis of *M. synoviae* infection.

Comparison of the Pathogenicity of Recent Isolates of *Mycoplasma synoviae* in Turkeys

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Department of Avian Medicine, University of Georgia

Systemic *Mycoplasma synoviae* (MS) infection was induced experimentally in commercial turkeys with recent MS isolates (K4822D and K4774J) from turkey breeder flocks. The virulence of each strain was compared by evaluating lesions, serological responses and MS re-isolation rates at 10 and 21 days post challenge and by comparing these results with those obtained from a known virulent isolate (K1968), another previously characterized field isolate (K4463B), and unchallenged controls. All strains caused lesions typical of infectious synovitis, but showed distinct differences in the extent of the gross and microscopic lesions and in the re-isolation rates from the tissues in turkeys. K1968 induced the most extensive lesions in hock and stifle joints and footpads, but strains K4822D, K4774J, and K4463B were all capable of inducing synovitis and were similar in virulence for synovial tissues. Very mild respiratory lesions were induced by all of the strains studied. All strains yielded strong positive serological responses in the serum plate agglutination test, the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay. MS was recovered from all turkeys challenged. It was concluded that these recent field isolates are virulent for turkeys and are probably incompatible with economic turkey production.

Clearance of *Mycoplasma synoviae* from the Upper Respiratory Tract by Local Treatment with Antibody and Tiamulin

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Mycoplasma synoviae (MS) is frequently implicated as sub clinical upper respiratory infections. Air sac infection occurs when it becomes combined with Newcastle disease, infectious bronchitis, or both. Chronic infection follows the acute phase and may persist for the life of the flock, and the antibiotic medication will not eliminate MS infection from the flock. Our previous study demonstrated that medicated MS commercial layers with tiamulin remained MS-PCR positive, which indicates the MS persistence stage. Two separate experiments were proved to eliminate MS from the upper respiratory tract by aerosolization with MS-antibody (Ab) or tiamulin solution to be inhaled by each chicken group. The efficacy of each treatment was validated by the recovery of MS by PCR assay. The MS chicken groups were obtained from a natural MS-infected flock. A pool of MS-Ab serum with an average titer of 3500, tested by ELISA (Kpl), was applied by aerosol three times at 72 hours intervals. The tiamulin solution were administered followed the above schema mentioned herein. The recovery of MS by a recommended PCR assay was performed in both experimental groups before and along the treatments. Histopathological picture of the upper respiratory tissues were examined to evaluate the changes in both groups. The results from the present study have been analyzed to conclude the effect of this novo bio-pharmacological strategy to clearance the MS from the upper respiratory tract of natural infected flocks.

Response to Treatment of Mycoplasmosis in Broiler Breeders Based on Evaluation by Pipped Embryo Analysis

Albert M. Payne, William A. Serna, and Tony Unandar

In the cases where the decision is made to implement a treatment program in an outbreak of mycoplasmosis, a question often remains as to the impact of the treatment program on vertical transmission. The use of pipped embryo analysis has been used to predict the shed rate and ultimately the prognosis for progeny from infected flocks.

This paper will briefly review the procedure for pipped embryo analysis and its use in determination of vertical transmission of mycoplasmosis. Data collected from both a controlled study and field experiences will show changes seen on pipped embryo analysis in response to various antibiotics.

Effect of Enrofloxacin and Tylosin on Protection Against MG Challenge in Birds Vaccinated with TS-11

Manuel Contreras, Rafael Fernandez, and Enrique Montiel
Merial Select

One hundred twenty day-old Leghorn pullets obtained from a commercial hatchery were divided into 7 groups that were used to determine the effect of enrofloxacin and tylosin on the protection level against challenge with R strain in birds vaccinatd with TS-11. The seven groups were kept in colony houses at the Merial-Select experimental farm in Gainesville, Georgia. Groups 1, 2, 3, 4, and 5 were vaccinated via eye-drop at 5 weeks of age with TS 11. Groups 6 and 7 were used as non-vaccinated controls. After vaccination with TS 11, group 1 was treated with enrofloxacin and group 2 with tylosin at 11 weeks of age and challenged at 15 and 20 weeks. Group 3 was treated with enrofloxacin and group 4 with tylosin at 14 weeks of age and challenged at 19 and 23 weeks. Seven and 14 days after challenge, the birds were scored to determine the presence of MG-R strain by manually expressing nasal discharge.

Tuesday, July 17, 2001
Session A

Reliable Virulence Measures for the Newcastle Disease Virus (NDV)

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Newcastle disease virus (NDV) strains produce in susceptible birds a continuum of clinical entities ranging from an inapparent respiratory infection to a fatal systemic disease. A reliable assessment of virulence of NDV is important in implementing control and eradication policies. Three virulence measures were compared: 1) chicken pathogenicity test, 2) intracerebral pathogenicity index (ICPI), and 3) amino acid profile at the fusion (F) protein cleavage site. Of these, the amino acid profile at the F protein cleavage site and ICPI were reliable measures of NDV virulence.

Virulence of Six Heterogeneous-origin Newcastle Disease Virus (NDV) Isolates for Domestic Chickens

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Department of Pathology, CVM, University of Georgia (Kommers, Brown)
Southeast Poultry Research Laboratory, USDA, ARS (King, Seal)

Objective - To determine the effect of chicken passage on the virulence of six NDV isolates.

Animals - Two-week-old SPF White Leghorns (WL) were used for virus passages. Intracerebral pathogenicity index (ICPI) was determined in day-old WL. Intravenous pathogenicity index (IVPI) values and intracloacal pathogenicity were determined in 6-wk-old WL.

Procedures - Four serial passages of NDV isolates from dove, pheasant, anhinga, yellow nape parrot, and chickens were completed. Tissues from inoculated birds (1st and 4th passages) were analyzed by histopathology and immunohistochemistry.

Pathotyping tests were performed with pre- and post-passaged viruses. Sequence analysis of the fusion protein cleavage site of each isolate was performed before chicken passages.

Results - Clinical disease was only observed with dove and pheasant isolates. Significant microscopic lesions were observed with anhinga, dove, and pheasant isolates. With dove isolate, lesions increased in severity from passage 1 to passage 4. Equally severe lesions were observed in birds inoculated with pheasant isolate during passages 1 and 4. Viral nucleoprotein was detected by immunohistochemistry among all affected organs. Based on pathotyping tests and sequence analysis, three isolates (chicken-LBM, yellow nape, and chicken-Australia) were characterized as low virulence and three (anhinga, dove, and pheasant), as virulent viruses. The dove isolate became highly virulent and the virulent pheasant isolate had ICPI and IVPI increase with passages.

Conclusions - Three of the viruses have significant pathogenicity for chickens and one of them had marked virulence increase after backpassages. These results demonstrate the high risk for commercial poultry represented by virulent NDV-infected wild and exotic birds.

Passive Immunization Protects Birds Following Challenge with Virulent NDV

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Newcastle disease (ND) is a highly contagious viral disease of poultry capable of causing high morbidity and mortality. The traditional strategy for controlling outbreaks of highly pathogenic ND is to eradicate exposed, or potentially exposed, flocks of birds. Although this strategy has proved successful, it typically results in large numbers of birds being euthanized. The environmental, economic and animal ethical issues of this strategy are of increasing concern. The objective of this study was to examine the potential for using passive immunization as an alternative strategy for controlling highly pathogenic outbreaks of ND. Here we determine the time interval between exposure and providing protection by administering anti-Newcastle disease virus (NDV) specific immunoglobulin subsequent to virulent NDV challenge.

Different groups of chickens were passively immunized (i.e. received Anti-NDV antibody) at various times with respect to challenge corresponding to 24 hrs. prechallenge, day of challenge, 1, 2, 3, 4, 5, 6, 8 and 9 days post challenge. HI titers were evaluated prechallenge prior to antibody injection and 24 hours post passive immunization. Serologic results indicated that all birds passively immunized had titers between 10 and 12 log2. The results of the challenge indicated that all birds that received passive immunization by 3 days following challenge were protected. Protection began to wane by 4 days post challenge and little (if any) protection was afforded by 8 days post challenge. In general, if birds were administered immunoglobulins prior to clinical signs of ND, they were afforded protection.

Newcastle Disease Vaccine Failure in Commercial Broilers

Dennis Senne, Bruce Seal, Daniel King, Eduardo Rivera, and Cesar Villarreal
USDA, National Veterinary Services Laboratory
USDA, ARS, Southeast Poultry Research Laboratory

Many commercial broiler companies are presently using off-label (reduced) dosages of live Newcastle disease (ND) vaccine to reduce adverse vaccine reactions. In the year 2000, a U.S.-based broiler company using this vaccination procedure was affected by an outbreak of exotic ND in Northern Mexico near the Texas border. Of the more than 90 flocks (>13 million birds) that were depopulated, >80% were owned by the U.S. based company. Epidemiologic studies showed that the use of off-label dosages of ND vaccine failed to protect broilers from clinical disease caused the exotic ND virus. A review of the outbreak, the ND vaccination program employed at the time of the outbreak, and characterization of the virus isolates will be presented.

Rapid Detection and Strain Differentiation of Infectious Bursal Disease Virus in Formalin-fixed, Paraffin-embedded Tissue

Mary J. Pantin, Thomas P. Brown, Mark W. Jackwood, and Heather Ainsworth
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Infectious bursal disease virus (IBDV) was detected by RT-PCR in formalin-fixed, paraffin embedded bursal tissues routinely sent for histological diagnosis of the disease. Rapid results were obtained using real-time RT-PCR and positive samples were detected by melting curve analysis. RT-PCR products were digested with restriction enzymes and IBDV strains were differentiated by melting curve analysis of resulting restriction fragments. This method is faster and simpler than the normal molecular diagnostic techniques such as RT-PCR/ RFLP, and has potential uses for epidemiological and diagnostic studies.

Detection of Single and Multiple Nucleotide Polymorphisms in Infectious Bursal Disease Viruses Using Real-time RT/PCR

Daral J. Jackwood and Susan E. Sommer
Food Animal Health Research Program, The Ohio State University/OARDC

The antigenicity and pathogenicity of infectious bursal disease virus (IBDV) strains are controlled by specific nucleotide sequences in the VP2 gene. Polymorphisms in these nucleotide sequences account for different antigenic and pathogenic viruses. Although nucleotide sequence analysis of RT/PCR products can be used to identify sequence polymorphisms, these procedures are not practical for routine diagnosis. Detection of single and multiple nucleotide polymorphisms has been reported using the LightCycler system (Roche Molecular Biochemicals). We have applied this technology to identify polymorphisms in the hypervariable region of the IBDV VP2 gene. The data generated were compared to the nucleotide sequences of the viruses examined. This technology has potential for becoming a useful diagnostic assay for the identification of specific mutations that are associated with IBDV antigenicity, pathogenicity and tissue tropism.

Pathogenicity of Recent IBDV Isolates Inoculated in Commercial Broilers Using in situ Hybridization

Alejandro Banda, Pedro Villegas, John El-Attrache, and Corrie Brown
College of Veterinary Medicine, The University of Georgia

Two infectious bursal disease virus (IBDV) field isolates from poor performance flocks were used to inoculate commercial broilers at 1 day and two weeks of age. *In situ* hybridization (ISH) was used to detect IBDV-RNA from the bursa of Fabricius, thymus, spleen, proventriculus, and kidney at 48, 96, 144, and 192 hours postinoculation. No clinical signs or mortality were observed in the chickens inoculated with both isolates. A slight decrease in bursal weight was observed when the chickens were inoculated at 1 day of age, whereas severe bursal atrophy was observed in the chickens inoculated at two weeks of ages. Differences of IBDV-RNA distribution in tissues were detected by ISH. Tissues of birds inoculated at two weeks of age exhibited a greater amount of IBDV-RNA positive cells in comparison with birds inoculated at one day of age and the bursa exhibited greater amount of IBDV-RNA positive cells when compared with other tissues of chickens inoculated at both ages.

Involvement of Cytokines in the Pathogenesis of Infectious Bursal Disease Virus (IBDV) in Chickens

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Infectious bursal disease virus (IBDV) induces massive infiltration of T cells in the bursa Fabricius at the site of virus replication. In situ hybridization and RT-PCR demonstrated that bursae of IBDV-infected chickens had upregulated expression of cytokines such as interferon (IFN)-y, tumor necrosis (TNF)-like factor as well as interleukin (IL)-2. When birds were depleted of functional circulating T-cells by thymectomy and cyclosporin A treatment (Tx-CsA), no upregulation of IFN-y and IL-2 was observed wihle the upregulation of TNF was not affected. The reduction in cytokine expression in Tx-CsA birds correlated with increased IBDV antigen load and a reduction in IBDV-induced bursal lesions in infected birds. These results indicate that T cells and T cell released cytokines such as IL-2 and IFN-y may be involved in the control of IBDV replication but also contribute to bursal depletion.

Cytokines Modulate IBDV Vaccination

Hung-Yueh Yeh, Silke Rautenschlein, and Jagdev M. Sharma
Veterinary PathoBiology, College of Veterinary Medicine, University of Minnesota

Modulatiuon of various avian cytokines in ovo vaccination is investigated. Chicken embryos at ED18 were immunized with IBDV vaccine in combination with avian cytokines. Several parameters including bursa and body weight ratio, antibody responses to IBDV, quanitification of gene expression will be determined. The results will be discussed later.

The Role of T-cells in Protection by Inactivated IBDV Vaccine

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Chickens with compromised T cell responsiveness were vaccinated with an inactivated infectious bursal disease virus (iIBDV) vaccine. The vaccine induced protective immunity in T cell-intact but not in T cell-compromised chickens. Immunocompromised chickens produced reduced levels of antibody because of compromised helper T cell function. Selective reduction in circulating CD8+ cells by anti-CD8 antibody treatment also had a profound effect on the efficacy of the iIBDV vaccine. Chickens with reduced circulating CD8+ cells showed reduced protection against a challenge with virulent IBDV whereas CD8-intact birds were fully protected. These results demonstrate that immunosuppressed animals with reduced T-cell function many respond poorly to inactivated vaccines.

Infectious Bursal Disease Virus Proventriculitis

Tami Kelly, Joe Giambrone, and Kalen Cookson
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Auburn University, and Fort Dodge Animal Health

Severe proventriculitis has been seen in broilers 3-5 weeks of age. The virology is negative for Reo virus. A variant IBDV has been isolated and typed to be a molecular group 6. Challenge studies with this virus do cause proventriculitis and of the available vaccines only 50% reduction of the proventriculitis could be obtained. Further studies are on going now and more details will be available in July.

Comparison of Monoclonals-Based AC-Elisa, RT/PCR-RFLP and Histology for the Diagnosis of IBDV

C.M. Lamichane
Synbiotics

AC-ELISA, PCR and histology methods were compared for the detection of IBDV in broiler flocks. Bursal samples were collected from broiler chicks at 17, 21, 25, and 28 days of age. The sensitivity and specificity of three methods will be discussed.

Pathogenesis of Chicken Infectious Anemia Virus: Studies on Latency

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Recent studies suggested that CAV may occur in a latent, often undetected state. It is hypothesized that latent virus can change to a productive infection caused by yet unknown activating factors. This study was undertaken to determine if 2 mg/kg dexamethasone injection, could initiate viral production in chickens known to be exposed, but testing negative using ante mortem testing methods. Results to date show that the stress of movement, artificial insemination, and dexamethasone did not result in formation of CAV antibodies. However, there is initial evidence by nested PCR that virus may have shed to embryos.

California 99 and Nebraska 95 strains of infectious bronchitis virus: Molecular and serological characterization

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A strain of IBV designated California 99 (CA99) has been repeatedly isolated from broilers in California. Reverse transcriptase-polymerase chain reaction and restriction fragment length polymorphism identification and characterization of the S1 gene for this isolate indicated that it was similar if not identical to the Nebraska 95 (NE95) isolate. Whereas the CA99 virus has only been isolated in California, NE95 has been isolated in the mid-western and southeastern U.S. Additional data on molecular and serological tests will be presented in an effort to further define the relationship of these viruses to other isolates of IBV.

Virological and Serological Identification of Infectious Bronchitis Virus (IBV) isolates known as DE-072 Variants

P. Villegas, M. Ruano, J. El-Attrache, and N. Ferguson
Department of Avian Medicine, University of Georgia

DE-072 IBV variants isolated from commercial broilers were classified into two separate groups. One group includes the proposed new serotype GA-98, while the other group is comprised of several distinct yet similar IBV isolates. These unique DE-072 IBV variants were passed in chicken embryos in an attempt to assess their pathogenicity. When inoculated in chickens, low chicken embryo (CE) passage isolates induced more damage in the trachea than high CE passage viruses. Chickens vaccinated with the high CE passage isolates provided protection against the homologous challenge, although lower protection was obtained against a DE-072 challenge. The DE-072 vaccine provided adequate protection against two of the three DE-072 variant strains tested.

The Pathogenesis and Genetic Characteristics of Nephropathogenic Infectious Bronchitis Viruses Isolated in the United States

Chang Won Lee, Deborah A. Hilt, Corrie Brown, and Mark W. Jackwood
The University of Georgia, Department of Avian Medicine

Four-day-old specific-pathogen free chickens were inoculated by eyedrop with four different strains (Gray, JMK, CV56b, and Wolgemuth) of infectious bronchitis virus. Birds were monitored clinically and euthanatized at 1,4, 7, and 14 days post-infection to collect tissue samples for histopathologic examination, *in situ* hybridization (ISH), and immunohistochemistry (IHC). Disease was most severe in chickens infected with Wolgemuth and no overt disease was observed with the other strains. Virus was isolated in the kidneys from Gray, CV56b, and Wolgemuth infected chickens. Further, interstitial nephritis was noticed in those chickens. However, viral nucleic acid and antigen was detected only with Wolgemuth infected kidneys by ISH and IHC. Our results indicate that the pathological changes in kidneys from chickens infected with Gray and CV56b may not result from the cytolytic action of the virus.

Protection Afforded By Commercial Vaccines Against the Nebraska 95 IBV Field Isolate

Ivan Alvarado, Pedro Villegas, John El Attrache, and John Glisson
Department of Avian Medicine, College of Veterinary Medicine, University of Georgia

An infectious bronchitis virus (IBV) from Nebraska was isolated from a commercial broiler farm exhibiting respiratory distress, inflamed tracheas, air saculitis and edematous lungs. After amplification by reverse transcriptase-polymerase chain reaction (RT-PCR), the S1 region exhibited a restriction fragment length polymorphism (RFLP) pattern different from the Massachusetts, Connecticut and Arkansas serotypes, but was identical to several IBV isolates obtained from California. The protection level conferred by IBV commercial vaccines against the Nebraska 95 IBV isolate was evaluated. Commercial Massachusetts-Connecticut and Massachusetts-Arkansas vaccines, with minimum titers of $10^{4.5}$ EID_{50}/ml, were used to vaccinate the chickens at one, ten or one and ten days of age. Birds were challenged at 27 days of age with the Nebraska 95 virus. Protection against the Nebraska 95 was determined by virus re-isolation from tracheas five days post challenge.

Prevalence of Infectious Bronchitis Virus During the Downtime period in Broiler Houses

Jaime Ruiz, John Glisson, and Karen Burns
The University of Georgia

Isolation and Characterization of Multiple Strains of Infectious Bronchitis Virus (IBV) from a Commercial Layer Farm

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Penn State University

Multiple strains of infectious bronchitis virus (IBV) were consistently isolated from three commercial layer flocks in a poultry farm by the use of SPF sentinel chickens and molecular assays. SPF white leghorn sentinel chickens were initially placed in the three houses of commercial layer flocks in early June 2000. Ten SPF sentinels in cages were placed in 5 different locations per house with 2 birds per cage. Tracheal and/or cloacal swabs were collected from all sentinels on a weekly basis for the first 5 weeks after placement, and thereafter, every 2-3 weeks. Infectious bronchitis virus (IBV) was successfully isolated from house 2 and 3 sentinel birds at 9 days after placement. These sentinels maintained shedding of IBV for a 4-week period. The sentinels were negative for IBV in two subsequent sample collections. The SPF sentinels in house 1 were negative for IBV during the first 4 weeks after placement. However, by the 5th week, IBV was isolated from sentinels placed in house 1, which remained positive for IBV during the following 6 weeks. Serotyping of the IBV isolates by monoclonal antibody (Mab)-based immunofluorescent and RT-PCR assays revealed that Connecticut, Arkansas, Pennsylvania nephropathogenic and Delaware strains of IBV existed at this poultry farm. A second group of SPF sentinels birds was placed in the three houses of commercial layer flocks in early November 2000. The same multiple strains of IBV were again isolated from the SPF sentinel birds.

The significance of the presence of numerous strains of IBV on this single premise will be discussed in relationship to the study of vaccination given and the continuing genetic variation encountered with this coronavirus in field situations.

Management of an Endemic Infectious Bronchitis 072-Variant in a Commercial Broiler Complex

Albert M. Payne

In the past two years, there has been an increase in the number of isolations of infectious bronchitis that are related but not identical to the 072 strain identified in the early 1990's. This case study will present a review of the history, clinical findings, diagnostics and prevention strategies used in a large commercial broiler complex in the southeastern US to address an endemic GA-98 Infectious Bronchitis outbreak.

Field Observations From the Use of a Vaccine Reaction Score System for IBV on Broiler Performance

Daniel Venne
Quebec, Canada

A vaccine reaction scoring system developed by Louise Defour-Zavala was used for a one year period during farm visits. Results will be compared between vaccinated and non vaccinated birds at day of age and three strains of day old bronchitis vaccine. Observations on management and final results will also be compared. The results show differences in peak reaction and possible interference with field vaccination. Seasonal and management factors having an effect on vaccine reaction will be discussed. Average score on 180 observations was 1.22 with a median of 0.5. Average vaccine reaction between 6 and 14 days of age for birds in a cleaned out barn on new litter and vaccinated at day old was 2.58 for vaccine A, 1.31 for vaccine B, 2.97 for vaccine C and 0.44 for non vaccinated birds. Reaction by age group was 0.07 for 0 to 5 days, 1.34 for 5 to 10 days, 1.93 for 10 to 15 days, 1.40 for 15 to 20 days, 2.23 for 20 to 25 days, 0.39 for 25 to 30 days and 0 for 30 to 35 days.

Tuesday, July 17, 2001
Session B

Evaluation of Avian *E. coli* Iss Monoclonal Antibodies

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Objective - The objective of this project is to determine the potential utility of Iss monoclonal antibodies (Mabs) as reagents for detection and study of virulent avian *Escherichia coli.*

Design - As part of an overall effort to explore the potential of *iss*-/Iss-based colibacillosis control strategies, Mabs to Iss have been generated and are being evaluated. These evaluations include using Mabs to screen *E. coli,* known to contain or lack *iss*, in order to determine if these Mabs have potential in detection protocols.

Sample Population - Well-characterized *E. coli* isolates from sick and healthy birds will be used to determine the utility of Iss Mabs for detection of virulent avian *E. coli.*

Procedure - Anti-Iss Mabs were prepared by fusing mouse splenocytes and myeloma cells, followed by selection and expansion of clones that recognized Iss. The Mabs were isotyped and evaluated for their specificity to Iss through western blotting. Currently, these antibodies are being used to detect Iss on *E. coli*, isolated from sick and apparently healthy birds.

Results and Conclusions - The results of this study will help us determine whether the anti-Iss Mabs will be useful in detecting and studying virulent avian E. coli.

Involvement of Iss in Avian *Escherichia coli* Virulence and Complement Resistance

Shelley M. Horne, Cathy W. Giddings, Richard E. Wooley, Penelope S. Gibbs, and Lisa K. Nolan
North Dakota State University (Horne, Giddings, Nolan)
College of Veterinary Medicine, University of Georgia (Wooley, Gibbs)

Objective - Improved control of avian colisepticemia.

Experimental Design - We have identified the *i*ncreased *s*erum *s*urvival (*iss*) gene, encoding the Iss protein, as a promising marker of virulence among avian *E. coli*. The *iss* gene of a virulent, complement-resistant avian *E. coli* isolate was mutated to determine whether *iss* contributes to virulence and resistance to complement activity.

Procedures - The *iss* gene was mutated in a plasmid vector temperature sensitive for replication, then electroporated into virulent complement-resistant avian *E. coli* isolates. Recombination of the mutation into the genome of the virulent avian *E. coli* isolates was promoted by incubation at 45 C. Mutation of the *iss* gene in each virulent isolate was confirmed by Southern analysis. The *iss*⁻ mutants and their isogenic parents were compared for complement resistance using flow cytometric and *in vitro* assays and for virulence in a chick embryo model.

Results - Southern analysis confirmed that the *iss* gene was mutated. Digests of genomic DNA from the *iss*⁻ mutants and their isogenic parents produced fragments of the expected sizes. Experiments to compare *iss*⁻ mutants with their isogenic parents for differences in virulence and ability to resist complement activity are in progress.

Conclusions - *iss* may be an important contributor to avian *E. coli* virulence.

The Role of *arsH* in Avian *Escherichia coli*

Penelope S. Gibbs, Richard E. Wooley, and John J. Maurer
Department of Medical Microbiology and Parasitology (Gibbs, Wooley)
Department of Avian Medicine (Maurer)
College of Veterinary Medicine, University of Georgia

Objective - Characterize a large molecular weight plasmid, pWT3, from untypable avian pathogenic *Escherichia coli* (APEC) isolate V-1.

Design - Sequence analysis of segments of pWT3 revealed the presence of a gene, *arsH*, not found in *E. coli* before. The function of *arsH* in APEC was studied via mutagenesis of *arsH* in experimental *E. coli* K12 strains, wild-type arsenite resistant *E. coli*, and in APEC isolates.

Procedure - Arsenite resistance assays are utilized to determine the resulting phenotypes of the mutagenized strains with respect to arsenite resistance.

Results - Certain *arsH* mutagenized isolates showed varying degrees of resistance to arsenite.

Conclusions - In *Yersinia enterocolitica*, *arsH* has been found necessary for arsenite resistance, but *arsH* was not required for expression of arsenite resistance genes from *Thiobacillus ferrooxidans*. Presently, results are inconclusive as to the role of *arsH* in arsenite resistance in *E. coli*.

Apralan® Soluble Powder for the Control of Colibacillosis-related Mortality in Growing Chickens

Bret Rings, Gregory M. Moore, and Alan Zimmermann
Elanco Animal Health

Three trials were conducted to confirm the efficacy of Apralan® Soluble Powder (apramycin sulfate) administered in the drinking water to growing chickens for the control of colibacillosis-related mortality caused by strains of *Escherichia coli* (*E. coli*) susceptible to apramycin sulfate. Apramycin was administered at levels of 0, 125, and 250 ppm *ad libitum* for 5 consecutive days after a determined level of disease in the flock had occurred. Analysis of the data from the three clinical trials demonstrates that the administration of apramycin in drinking water at 125, and 250 ppm for at least 5 consecutive days significantly reduced ($p < 0.004$) colibacillosis-related mortality when chickens were treated in the early stages of a colibacillosis outbreak.

Characterization of Recent *Pasteurella multocida* Isolates from the West and Midwest

Elisabeth J. Angrick, Pyone Pyone Aye, and Teresa Y. Morishita

Pasteurella multocida isolates from commercial turkey operations in one western state (1996-7), one north central state (1997), and one northeast state (1997-2001), were characterized by biotyping, serotyping, DNA fingerprinting, antibiotic susceptibility patterns, and plasmid analysis. Different serotypes, DNA fingerprint profiles, and antibiotic susceptibility patterns were identified within and among the different geographical areas. Relatively few of the isolates harbored plasmids.

Evaluation of the Effect of Heating on Oil Emulsion *Pasteurella multocida* Bacterin on Reducing Tissue Reaction Without Affecting Immunity

Karen Burns, Jaime Ruiz, and John Glisson
The University of Georgia

Oil emulsion vaccines create a long duration of immunity in broiler breeders and layers. If injected in the breast muscle, problems with intramuscular lesions arise at the spent fowl plant. This study evaluates the efficacy of heating an oil emulsion bacterin to reduce intramuscular lesions. The vaccine was heated to 100°F and injecting intramuscularly in the superficial pectoral muscle. Two vaccinations were given 4 weeks apart. Challenge with X-73 strain of *P. multocida* evaluated the efficacy of immunity between groups. Serologic response to vaccination was evaluated using ELISA. Breast lesions were scored using an established grading system.

Lesions Associated with an Accidental Head Injection with a Live Fowl Cholera Vaccination

Robinette A. Gilbert

Large subcutaneous lesions were evaluated on broiler breeders coming from one common pullet flock. The pullets were placed on two breeder farms. The serviceman reported that there were approximately 200 affected birds on each breeder farm. The lesion consisted of a large subcutaneous core, which was consistently confined to the head. No lesions were detected in the joints, neck, waddles, ovaries, or liver. Most of the time the lesion was confined to the crown of the head. However, drainage into the sinuses was not uncommon. The lesion appeared to originate internally and not externally as with trauma or pecking. The appearance of the lesion and the consistent location led to the suspicion that the flock had been accidentally injected in the head with the live cholera vaccine. A trial was conducted to duplicate this and confirm our suspicions.

Extraction, Preparation, and Efficacy of *Pasteurella multocida* Capsule and Outer Membrane Proteins in Pekin Ducks

Amna B. El Tayeb, Jessie I. Price, and Michael T. Collins
Department of Veterinary Preventive Medicine, The Ohio State University
Department of Veterinary Science, The University of Wisconsin-Madison
USGS Biological Resources Division, National Wildlife Research Center
Department of Pathobiological Sciences, The University of Wisconsin-Madison

Separation of *P. multocida* type A:1 capsular and outer membrane proteins (OMP) was achieved. The OMP was extracted by treatment of whole cell lysate with sarkosyl. The capsular polysaccharide was extracted by 2.5% NaCl and 1% cetylpyridinium. The protective efficacy, dose response, and serological response for both capsular and OMP were evaluated and the results were compared to those of the standard bacterin. The *P. multocida* capsular vaccine was the least immunogenic, only 50% protection from challenge with virulent *P. multocida* was achieved at 16 weeks post-vaccination with no protection at 26 weeks post-vaccination. All ducks vaccinated with OMP and standard bacterin withstood the challenge with virulent *P. multocida*. The serum antibody response, as measured by indirect ELISA, was higher for the ducks vaccinated with OMP and bacterin. Administration of a second dose had dramatic effect on the serum antibody response regardless of the vaccine used. Vaccines with a higher antigen content evoked a higher serum antibody response.

Factors Affecting Pathogenesis of Avian Pneumovirus in Turkeys

Faris F. Jirjis, Sally L. Noll, David A. Halvorson,
Kakambi V. Nagaraja, Alberto Back, and Daniel P. Shaw
College of Veterinary Medicine, University of Minnesota

Four and nine-week-old turkey poults were inoculated with cell culture propagated avian pneumovirus (APV) into each conjunctival space and nostril, followed by inoculation with *E. coli, Bordetella avium, or Ornithobacterium rhinotracheale* 3 days later. Clinical sings were evaluated on days 3, 5, 7, 9, 11, 13 post-inoculation. The poults were euthanized on days 6, 10 and 14 post-inoculation and blood and tissues were collected. The poults that received APV and bacteria developed more severe clinical signs and pathological changes than uninoculated controls or those birds given single infection of APV or bacteria, particularly when *Bordetella avium* was involved in the group of 9-week old poults. *Bordetella avium* was recovered from tracheas and lungs of several birds that were inoculated with the APV and *Bordetella avium*. Viral antigen was detected by immunohistochemical staining of the upper respiratory tract from APV alone and co-infected groups on days 6 and 10. Viral antigen was also detected in the nasal turbinate and lungs of the group inoculated with APV and *Bordetella avium* on day 14 PI.

Neonatal Avian Pneumovirus Infection in Commercial Turkeys

David Halvorson, H.J. Shin, F.F. Jirjis, M.C. Kumar, M.K. Njenga,
D.P. Shaw, S.L. Noll, and K.V. Nagaraja
The University of Minnesota

Immune Response of Turkeys Following Intranasal Vaccination with BPL-inactivated Avian Pneumovirus and Live-Virus Challenge

Darrell Kapczynski and Cassandra Smith
USDA, Southeast Poultry Research Lab

Avian pneumovirus (APV) is an emerging disease in the U.S. poultry market. To investigate immunity against APV, turkeys were immunized intranasally with BPL-inactivated APV (Minnesota 1a) and challenged with homologous virus. Based on clinical signs, vaccinated birds exhibited increased protective immunity compared to non-vaccinated birds. However, RT-PCR analysis and virus isolation indicated that virus was detected in both vaccinated and non-vaccinated groups up to 2 weeks post-challenge. Antibodies against APV increased in all challenged groups throughout the course of the experiment. Mean serum IgG levels were decreased in APV challenged birds compared to controls, possibly indicating immunosuppression by the virus.

Effect of Fermentable Diets on Enterocyte Maturation and Turkey Coronavirus Infection

F. William Pierson, Chanin Tirawattanawanich, and Calvert T. Larsen
Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech

Objective - Investigate the effect of dietary fiber on intra-luminal short chain fatty acid (SCFA) production, enterocyte maturation, and severity of coronavirus (TCV) infection in turkeys.

Design - Completely randomized block design with sub-sampling.

Animals - Commercial turkeys were used in 3 blocks of 75 birds with 25 birds per treatment.

Procedure - At 1 day-of-age, poults were provided with cellulose fortified (C), guar gum fortified (G) or basal diets (B) *ad libitum* for 8 days. On day 9, 10 birds from each treatment were euthanized and sampled. Cecal SCFA's (C_2-C_5) were quantified by gas chromatography. Intestinal villin expression levels were determined by immunoblot. Remaining poults were challenged with TCV and the severity of infection was evaluated 24 hours later using immunohistochemistry.

Results - Poults receiving the diet G produced higher levels of all SCFA's when compared with C or B ($p < 0.05$). Acetate and butyrate levels were higher in poults fed C when compared with those fed B ($p < 0.05$). Villin expression was increased in poults fed G and C when compared to B ($p < 0.05$). The severity of TCV infection was highest in poults fed B and lowest in those fed G.

Conclusions - Data suggest that fermentable diets promote the production of SCFA's. Related increases in villin expression, which denote enhanced enterocyte differentiation and maturation, may correlate with more rapid replacement of TCV infected cells. This provides evidence that dietary fiber may be of value in shortening the course of TCV induced enteritis.

Effects of Turkey Coronavirus Infection on Turkey Breeder Hen Performance

David V. Rives and James S. Guy
Prestage Farms and North Carolina State University

A flock of turkey breeders experienced an episode of flushing beginning at 42 weeks of age. The flushing was accompanied by a period of decreased feed consumption (up to 26%) and decreased egg production. Cull eggs increased over 100%, and settable eggs leaving the farm dropped by 42%. Seven-day fertility also apparently decreased (over 7%), but was more likely an increase in early dead embryos due to nutrient malabsorption and decreased shell quality. Acute and convalescent sera demonstrated seroconversion to turkey coronavirus. Previous flocks seroconverting to coronavirus prior to the onset of egg production experienced no such production problems.

Production Effects of Exposure to Coban® in Turkey Breeder Hens in Production that had Previous Exposure to Coban® during Rearing as Breeder Replacements

James D. Trites and Ronald L. Lippert

Coban® (monensin sodium) was fed to a flock of 4,500 fifty-one week old turkey breeder hens in egg production. The breeder hens had been previously exposed to Coban as growing breeder replacements. Production parameters were compared to a sister flock located beside the test barn. The treatment flock was provided access to feed with 72g/ton of Coban in it for five consecutive days. A small increase in mortality (1.8%) above the sister flock was observed for 6 days. It started on the fifth day of administering the Coban feed and ceased five days after it was removed. Egg production during the five days the turkey breeders had access to the Coban feed showed no change in pattern when compared to the sister flock. However, in the 6 days that followed the administering of the Coban feed, egg production decreased approximately 3% when compared to the control production flock. The only clinical signs were a few birds that were unable to walk properly and the above normal mortality for 6 days. The clinical picture seen in this study was different from previous published reports on Coban exposure in turkey breeders. The exposure of an approved growing turkey Coban dose, as opposed to the high Coban levels reported in the previous publications, may account for the reduction in the clinical conditions of this study. It is also hypothesized that having previous exposure to Coban as growing breeder replacements may provide some protection against later erroneous exposure to Coban.

Pseudoaspergillosis in Young Turkey Poults Caused by *Staphylococcus aureus*

James Barton
Cargill, Inc.

Aspergillosis is a fungal disease commonly identified in young turkeys. It has a unique presentation, and entry-level poultry veterinarians should recognize aspergillosis from gross lesions alone. This case report describes an infectious disease caused by *Staphylococcus aureus* that even experienced poultry veterinarians would easily confuse with mycotic pneumonia caused by Aspergillus sp. Gross lesions, histopathology, bacteriology, treatment, and epidemiology will be presented. The presenter will discuss management factors that increase the risk of occurrence and spread of this unique presentation of pulmonary *Staphylococus aureus*.

Efficacy of Fenbendazole Against Round Worms in Commercial Turkeys

Dr. Daniel Karunakaran

Gastrointestinal nematodes are responsible for significant annual losses to the poultry industry in productivity and revenues. This is particularly true with regard to turkeys which by virtue of their extended grow out period (14-20 weeks) are subject to potentially high parasite loads. The detrimental effects of roundworm infestation include decreased rate of gain, feed conversion, general failure to thrive and in some cases, transmission of other infectious agents.

Recent studies have shown Fenbendazole to be effective against common gastrointestinal nematodes of turkeys when fed at approximately 16 PPM in feed for 6 days.

Results from Pen trial and commercial turkey flocks will be presented.

Reduced Two-week Mortality in Turkey Poults Following Administration of Tylosin Soluble

Robert D. Evans and Daniel Karunakaran
Elanco Animal Health and Shadybrook Farms Inc.

The purpose of this study was to determine if administration of tylosin soluble to one-day old poults would reduce two-week mortality. Hen-poult placements during four-week periods in 1998 and 1999 were randomly assigned to either a non-treatment or treatment group. Poults in the non-treatment group were provided *ad libitum* access to un-medicated water and in the treatment group were provided *ad libitum* access to water containing 2 g/gal tylosin for 48 hours. Housing and nutrition were within company guidelines for this complex and all birds were provided a similar standard of care. Two-week mortality was determined from daily mortality tabulations and used to determine significant ($p \leq 1.0$) difference using the student's two-tailed t test. Forty-two flocks (47%) were in the non-treatment group while 47 (53%) were in the treated group in 1998. In 1999, 43 flocks (45%) were in the non-treatment group while 53 (55%) were in the treated group. Reduced mortality was noted in the tylosin treated group each year. Two-week mortality in turkey poults may be reduced when tylosin is administered via the drinking water for the first 48 hours following placement.

Turkey Enteritis - Gross and Histologic Evaluations from the Field

Timothy Cummings

Over a period of time, necropsies of brooder age turkeys (2-7 weeks) from a turkey integrator were performed with lesions recorded in a data base for evaluation. In the course of this project, certain gross enteric conditions were noted which were documented via pictures with a digital camera. Sections of the same intestinal tissues were then processed for histologic evaluation and stained for bacteria. Matching the gross presentations with histologic/stained findings provided some interesting findings. This information will hopefully provide some help to field technical personnel in assessing turkey enteric conditions.

Characterization of a Reovirus Isolated from PEMS Causing Liver Lesions

K.A. Schat, Priscilla O'Connell, Cherilyn Heggen-Peay, and M. Qureshi
Cornell University (Schat, O'Connell), North Carolina State University (Heggen-Peay, Qureshi)

Poult enteritis and mortality syndrome (PEMS) is a multifactorial disease in turkey poults. Several viruses have been isolated from PEMS material including astroviruses. Inoculation of any of these viruses has not yet resulted in the complete reproduction of the disease. Recently, we isolated a reovirus from PEMS material that causes severe reduction in relative liver weight in experimentally infected poults. Preliminary studies indicate that this reovirus may be smaller than expected for reoviruses.

The Pathogenesis of Turkey Astrovirus Infection in Poults

Erica L. Behling-Kelly, Stacey Schultz-Cherry, Matt Koci, Laura Kelley, and Corrie Brown
University of Georgia, College of Veterinary Medicine (Behling-Kelly, Brown)
USDA, ARS Southeast Poultry Research Laboratories (Schultz-Cherry, Koci, Kelley)

Objective - To determine the pathogenesis of turkey astrovirus (TastV) infection in poults using riboprobe in-situ hybridization (ISH).

Design - Poults were infected experimentally and then euthanized in a 9 day time course. Tissues were examined for pathological changes and viral replication was detected in situ using a ribroprobe specific for the capsid gene of TastV.

Animals - Twenty-seven coronavirus-negative 7 day old poults were housed in Horsfall units. Of these, 21 poults were infected and 6 served as controls.

Procedure - Poults were orally infected with a bacteria-free intestinal homogenate harvested from TastV infected embryos. Birds were euthanized at 1,2,3,4,5,7 and 9 days post infection (dpi) with collection of tissues in formalin. In situ hybridization was used to identify cells that supported viral replication.

Results - Infected birds became clinically ill with diarrhea. By ISH, viral replication within the intestinal villus epithelium was detected at 1 dpi, peaked at 5 dpi, and was no longer detectable at 9dpi. Viral replication was not detected in any extra-intestinal tissues.

Conclusions - TastV causes a mild enteric infection in poults characterized by viral replication in the distal small intestine with orad and aboral extension to the duodenum and colon.

Wednesday, July 18, 2001
Session A

A Recombinant Fowl Pox Virus Containing IBV-S1 Gene and its Potential for a Vaccine

Mazhar Khan, X. Wang, W. Schnitzlein, and Deoki Tripathy
University of Connecticut and University of Illinois

Recombinant fowlpox virus (rFPV) containing a cDNA copy of the S1 gene of infectious bronchitis virus (IBV) was constructed and its imunogenicity and vaccine potential were evaluated. The recombinant fowlpox virus elicited anti-IBV protective immunity was indicated by the manifested, relatively mild clinical signs of the disease, decreased titers of challenge virus, and less severe histological changes of tracheas in virulent IBV-challenged chickens. In contrast, chickens immunized with either recombinant or parental FPV were resistant to a subsequent, virulent FPV challenge.

Homologous Fowlpox Virus Derived Promoters for the Development of Recombinant Vaccines

Viswanathan Srinivasan, William M. Schintzlein, and Deoki N. Tripathy
Department of Veterinary Pathobiology, University of Illinois at Urbana-Champaign

Fowlpox virus (FPV) is being used extensively to generate effective recombinant vaccines for the poultry industry. For efficient expression of foreign genes, appropriate promoters capable of directing the production of sufficient antigen to induce both humoral and cell mediated immunity are required. Towards the generation of FPV vectors, we have identified six strong FPV promoters including a novel bi-directional one. Analysis of promoter strengths both at transcriptional and posttranscriptional level revealed that A-type inclusion body protein gene promoter and bi-directional promoter are strong in dictating a high level of expression. Current efforts are being directed towards demonstrating the utility of these promoters in the generation of polyvalent vaccines.

Efficacy and Safety of a Recombinant Fowl Pox Virus Containing Laryngotracheitis Genes

Kristi M. Moore, Jennifer R. Davis, Yoshinari Tsuzaki,
David R. Hout, Motoyuki Esaki, Takashi Okuda, and Joan D. Leonard
Biomune Company

Objective - The objective of these studies was to construct a safe and efficacious recombinant fowl poxvirus vaccine containing laryngotracheitis virus (LTV) genes that does not revert to virulence or spread to other chickens.

Design - Molecular techniques were used to construct and verify a recombinant fowl poxvirus vaccine containing LTV genes (rFPV/LT). Trials were conducted to evaluate efficacy and safety of this vaccine.

Animals - Chickens, various avian species, and mammalian species.

Procedure and Results - The recombinant rFPV/LT vaccine was constructed by inserting protective LTV genes into the genome of fowl poxvirus (FPV). These gene inserts were stable in the FPV genome when evaluated by gene verification and expression after multiple passages *in vitro*. Following USDA APHIS guidelines, rFPV/LT was efficacious in chickens against challenge with LTV and FPV. The rFPV/LT recombinant vaccine did not revert to virulence after multiple backpassage and did not spread to non-vaccinated chickens. In comparison to the parent FPV, rFPV/LT and the parent FPV had similar stabilities in the environment, similar tissue tropisms, and similar virus isolations in avian and mammalian species evaluated.

Conclusions - The rFPV/LT recombinant vaccine is stable *in vitro* and *in vivo*. It is safe for use in chickens and similar to the parent FPV in the other species evaluated. The rFPV/LT recombinant vaccine is efficacious against challenge with LTV and FPV.

Analysis of *Mycoplasma gallisepticum* genes expressed by a fowl poxvirus vector

Yoshinari Tsuzaki, Jennifer R. Davis, David R. Hout, Shuji Saitoh, Motoyuki Esaki,
Takashi Okuda, Ayumi Fujisawa, Kristi M. Moore, and Joan D. Leonard
Biomune Company

Objective - Two protective antigen genes from *Mycoplasma gallisepticum* (MG) were isolated, and a recombinant fowl poxvirus vaccine expressing the genes was developed (rFPV/MG). The objectives of this research were to characterize the two MG antigens and to evaluate the safety and efficacy of rFPV/MG.

Design - MG growth inhibition test was conducted to characterize the MG antigens. MG challenge trial and various safety trials were conducted to evaluate the safety and efficacy of rFPV/MG.

Animals - Chickens and various avian species were used for the safety and efficacy trials of rFPV/MG.

Procedure and Results - Antibodies against the MG antigens inhibited MG growth significantly, suggesting those antigens were important for MG growth. Following USDA APHIS guidelines, the rFPV/MG vaccine was efficacious in chickens challenged with MG and FPV. The rFPV/MG vaccine did not revert to virulence or spread to other chickens. The infectivity to other avian species or mammalian cell lines, the tissue tropism, and the stability in the environment of rFPV/MG were similar to parent fowl poxvirus. The genome structure was stable after multiple passages *in vitro* and *in vivo*.

Conclusions - The rFPV/MG vaccine expressing two MG genes, which are considered to involve MG growth, is safe, stable, and efficacious against challenge with MG and fowl poxvirus.

Diseases and Lesions in the Integument

Richard Julian
Ontario Veterinary College

I have recently produced 3 slide study sets on cardiovascular disease in chickens and turkeys. I have many good pics of skin lesions and have offered to make up a study set on "lesions in the integument". I would like to show the set I have at the AAAP meeting and ask for additional and better slides from other Pathologists. I also want to ask for help to write and organize the text, since integument is not one of my specialties.

Role of Management in Control of fowl Pox in Laying Chickens

Radivoje Spasojevic

Genetic and Antigenic Characterization of a Poxvirus from Ostrich

Deoki N. Tripathy, Tae-Joong Kim, H.L. Shivprasad, and Peter R. Woolcock
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana
California Veterinary Diagnostic Laboratory System, Fresno Branch

Poxvirus infections are common in domestic and wild birds. In this study, genetic, antigenic and biological characterization of an avianpox virus isolated from the cutaneous lesions in ostriches was done. The ostriches from which poxvirus was isolated had been placed on the premises where turkeys had been raised. Antigenic and genetic studies did not reveal any significant differences between ostrich isolate and fowlpox virus. Susceptible chickens immunized with the ostrich virus were protected when challenged with fowlpox virus. Thus, the ostrichpox virus had biological, antigenic and genetic properties of fowlpox virus.

Infectious Coryza Outbreak in a Large Egg Layer Complex

H.M. Opitz
University of Maine

Infectious coryza caused by *Haemophilus paragallinarum* has not occurred in commercial poultry in the New England/New York area for more than 10 years. A case report will be presented of an unusually severe outbreak of Infectious Coryza which spread rapidly through several layer complexes with about 3 million white and brown egg layers. Besides the typical signs of Infectious Coryza an atypical severe muco-fibrinous-hemorrhagic tracheitis was observed. Egg production dropped between 20 to 80% and mortality exceeded 10% in several flocks. Viral infections were not involved. Haemophilus was often isolated in pure cultrue from affected hens. A case report describing the clinical signs, gross and histopahology, epidemiology, differential diagnosis and control will be presented.

A Caveat in FPV Vaccines and Their Derived Recombinants, A Need for Better Vaccine

Pratik Singh, William M. Schnitzlein, and Deoki N. Tripathy
Department of Veterinary Pathobiology, University of Illinois at Urbana-Champaign

We have previously reported that reticuloendotheliosis virus (REV) sequences are present in the genomes of fowlpox virus (FPV) isolates endemic in the United States. These FPV field isolates are heterogeneous populations in that the primary component contains a nearly intact provirus, whereas individuals comprising the minority have retained one of the two distinct-sized portions of the REV long terminal repeats (LTRs). Similar LTR remnants, but not REV coding regions, have been detected in the DNAs of commercial vaccines. Since to date a FPV isolate lacking any integrated REV sequences or having a different REV insertion site has not been identified, this site-specific acquisition of REV may confer a replicative advantage to FPV. The vaccines containing remnant LTRs may offer potential sites for REV integration. Studies are in progress to determine (1) the develop FPV lacking REV sequences (2) the role of integrated REV in FPV pathogenesis and (3) to develop a recombinant virus which can offer protection against FPV and REV.

Immunogenicity of Recombinant Plasmid DNA Expressing the VP2 Capsid Protein Gene of Infectious Bursal Disease Virus and Chicken Interleukin-2

Diane J. Hulse and Carlos H. Romero
University of Florida, College of Veterinary Medicine, Department of Pathobiology

Much attention has recently been given to the use of naked DNA molecules for vaccination against pathogenic viruses and to the utilization of immune cytokines as potentiators of the protective immune response. We have constructed recombinant plasmid DNA molecules that express the VP2 capsid protein gene of infectious bursal disease virus (IBDV) and the chicken interleukin-2 (Ch IL-2) gene in avian and mammalian cells. These recombinant DNA molecules were injected into chickens, individually or combined, and evaluated for their *in vivo* expression and protective ability against a homologous challenge with virulent IBDV. A third group of chickens was vaccinated with a commercial live attenuated vaccine. A fourth group was vaccinated with the "empty" parental plasmid. Protective immunity parameters measured were: bursa/body weight ratios, presence of viral RNA, histological lesions in the bursa of Fabricius and the development of clinical signs and mortality. Expression of the IBDV VP2 protein was demonstrated in the muscle of chickens injected 14 hours after injection. Preliminary data indicate that 4 days after challenge, chickens injected with the attenuated vaccine and a combination of IBDV VP2/Ch IL-2 plasmids had bursa/body weight ratios similar to those of unvaccinated unchallenged chickens. The bursa/body weight ratio was severely affected in chickens vaccinated with IBDV VP2 plasmid DNA alone and in chickens vaccinated with the parental plasmid DNA. This "protection" was not observed in any of the groups when the bursa/body weight ratios were evaluated at 7 and 14 days after challenge. Effective experimental DNA immunization of chickens and the potentiating effect of immune cytokines is presently hampered by the lack of knowledge related to dose, frequency of vaccination and balance between the viral gene DNA and avian cytokine gene DNA.

DNA Vaccination with Plasmids Containing Various Fragments of Large Segment Genome of Infectious Bursal Disease Virus

Ching Ching Wu, Hua Chen Chang, and Tsang Long Lin
Department of Veterinary Pathobiology, Purdue University

The present study was undertaken to determine the importance of including VP2 gene of the large segment genome of infectious bursal disease virus (IBDV) in the DNA vaccine for protection against infectious bursal disease (IBD) in chickens. Different fragments of the large segment gene of IBDV were successfully amplified by reverse transcription-polymerase chain reaction (RT-PCR) followed by cloning into an eukaryotic expression vector as DNA vaccines. Chickens (one day old) were intramuscularly injected with the individual plasmid, challenged with IBDV at 21 days old, and examined for 10 days. Chickens receiving the plasmids containing VP2 genes did not have clinical signs, mortality, and bursal atrophy and were effectively protected against IBDV infection. The results indicate that inclusion of VP2 gene in the plasmid DNA is essential in achieving effective protection mediated by DNA vaccination against IBDV infection in chickens.

Determination of the Most Effective Route for *in ovo* Delivery of DNA Vaccines

Lenita C. Moura, S. Elankumaran, G.L. Oshop, D. Bautista, J.D. Wilson, and R.A. Heckert
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland

In ovo vaccination is being used in more than 80% of all broilers produced in the USA. DNA vaccination has been evaluated for protection of chickens against Newcastle disease, avian influenza, infectious bursal disease, and coccidiosis. We have evaluated several different routes of *in ovo* delivery of plasmid DNA. Using a green fluorescent protein (GFP) reporter gene, we have determined the most effective route to deliver plasmid DNA to the developing chicken embryo.

In ovo Nucleic Acid Immunization of the chicken against Infectious bursal disease and Newcastle disease

Gretchen L. Oshop, S. Elankumaran, V. Vakharia, J.D. Wilson, L.C. Moura,
D.A. Bautista, and R.A. Heckert
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park

Nucleic acid vaccines represent a powerful new approach to control infectious agents. This novel technique involves the direct inoculation of purified DNA and has been shown to induce an immune response in several different species, including chickens. Eukaryotic expression vectors containing gene(s) encoding foreign protein(s) can be used as vaccines. Uptake of the vaccine DNA by host cells and subsequent foreign protein expression initiates the immune response. Although there have been a few reports of DNA vaccines being used in the avian species, few have involved the delivery of the vaccine *in ovo*. We have delivered plasmid DNA encoding genes for Infectious Bursal disease and Newcastle disease viruses to late stage developing chicken embryos by a modification of the widely practiced *in ovo* vaccination technique. We have evaluated the immune responses to the DNA vaccine in the newly hatched chicks. Using this technique, we have been able to demonstrate insert-specific mRNA production from the plasmid in several different embryonic tissues. Further *in ovo* immunization trials are underway.

Wednesday, July 18, 2001
Session B

The Concurrence of *Staphylococcus aureus* with Broiler Ascites

Robert Keirs, Chinling Wang, and Donnie Zumwalt
Mississippi State University

In the month of October broiler chicks from a single breeder flock were the first broilers placed in a new computerized controlled and monitored tunnel ventilated facility of 27,500 capacity. Litter was new and of two types. Mortality increased to 34 day 19, 47 day 24, 80 day 30 and 104 day 37. Necropsies of dead birds on days 24 and 30 revealed predominately ascites and gizzard erosion with heart and liver cultures on day 30 of Staphylococcus aureus. Cultures of hearts and livers and lungs of live birds with ascites on day 34 also revealed Staph. aureus.

Four-year Summary of the Disease Incidence of Broiler Breeders in North Alabama

Francene S. Van Sambeek
Hinton Mitchem Poultry Diagnostic Laboratory, Alabama

Objective/Design - Case report on disease incidence

Population - Multiplier, commercial, broiler-breeder flocks, which were older than 22 weeks of age, submitted to the Hinton Mitchem Poultry Diagnostic Laboratory, located in Hanceville, Alabama for mortality and disease diagnosis. The years referenced are fiscal years (FY) that begin in October of the preceding year and end in September of the designated year.

Procedure - Numbers reported indicate were a primary disease diagnosis was made.

Results/Conclusions - Primary viral diseases consisted of 1) Avian leukosis virus, subgroup J, found in 193 flocks with the majority of the cases diagnosed in FY98 and FY99. 2) Infectious bronchitis virus, serotype Georgia 98 found in 3 flocks. 3) Vaccinal infectious laryngotracheitis was diagnosed in 1 flock. Primary bacterial diseases consisted of 1) Bacterial peritonitis with *Escherichia coli* isolated confirmed in 90 breeder hen flocks, usually occurring between 26 to 32 weeks of age. 2) Staphylococcal synovitis confirmed in 70 cases. 3) Fowl cholera isolated in 65 cases. 4) *Mycoplasma synoviae* positively identified in 60 flocks with *M. gallisepticum* found in 9 flocks. 5) Infectious coryza identified in three flocks. Parasitic diseases primarily consisted of two etiologic agents: Capillariasis in 13 flocks and Northern fowl mites in 8 broiler breeder flocks.

Production Loss and Mortality in Broiler Breeders from Suspected Salinomycin Toxicity

Charles S. Roney
Sanderson Farms

Salinomycin is an ionophore that is commonly used to control coccidiosis in broilers and broiler breeders. Reports of toxicity in commercial chickens are rare. This case report describes a 40% mortality rate with resultant loss of egg production in a house of 40 week old broiler breeders where the feed was accidentally contaminated with salinomycin. Lesions, signs, pathology and contamination levels will be discussed as well as a brief overview of previously reported ionophore toxicities in commercial broiler breeders.

Antibiotic Sensitivity Profiles of *E. coli* Isolates from Commercial Broiler Chickens Submitted to the Salisbury Animal Health Laboratory from 1998-2000

Nathaniel L. Tablante and Fidelis N. Hegngi
Virginia-Maryland Regional College of Veterinary Medicine (Tablante),
Maryland Department of Agriculture, Salisbury Animal Health Laboratory (Hegngi)

Objective - To analyze the antibiotic sensitivity profiles of *E. coli* isolates from clinically ill broilers submitted to the Salisbury Animal Health Laboratory (SAHL) from 1998 to 2000.

Design - Retrospective study

Animals or Sample Population - All *E. coli* isolates from broiler specimens submitted to the SAHL and tested for sensitivity to a panel of antibiotics from 1998 to 2000 were included in this study.

Procedure - From 1998 to 2000, the Maryland Department of Agriculture and the Virginia-Maryland Regional College of Veterinary Medicine recorded (using database program) the antibiotic sensitivity profiles of *E. coli* isolates from clinical ill broiler chickens submitted to the SAHL. The antibiotics tested were enrofloxacin, sarafloxacin, erythromycin, gentamicin, neomycin, penicillin, tetracycline, trimethoprim, and ceftiofur sodium. The data was exported to a spreadsheet program and analyzed using a statistical software program.

Results - Data analysis showed an increasing sensitivity to enrofloxacin, sarafloxacin, gentamicin, and ceftiofur sodium. Sensitivity to neomycin decreased slightly from 75% in 1998 to 68% in 2000. Ninety percent or more of the *E. coli* isolates were resistant to erythromycin, penicillin, and tetracycline. However, 68% to 72% of the isolates were sensitive to trimethoprim.

Conclusion - While fluoroquinolones such as enrofloxacin and sarafloxacin have shown good efficacy against *E. coli* infections in poultry, approval of these antibiotics for poultry use was recently withdrawn by the Food and Drug Administration. Based on data collected to date, trimethoprim appears to be a suitable alternative for the treatment of *E. coli* infections in broiler chickens on the Delmarva peninsula.

Rofenaid® Disk-Diffusion Assay Results

Steven R. Clark

Sulfadimethoxine plus ormetoprim (ROFENAID®, Alpharma Inc., Fort Lee, NJ, USA) is available as a medicinal feed additive for use in broilers and turkeys. A study was conducted to assess the antimicrobial activity of sulfadimethoxine plus ormetoprim against *Pasteurella multocida* (ATCC #43137) and *Escherichia coli* (ATCC #8739). The test products were 6.4-mm filter paper disks impregnated with sulfadimethoxine plus ormetoprim. Disk-diffusion assays were performed and zones of inhibition recorded. Disk-diffusion assays indicate that Rofenaid® is antimicrobially active against *P. multocida* at 15.0-17.9 mm zone size. The zone size for *E. coli* was 23.9-24.3 mm. Results showed that sulfadimethoxine plus ormetoprim exhibited antimicrobial activity against the organisms tested.

Pathologic Characterization of Four Michigan Infectious Laryngotracheitis Virus Isolates

Richard M. Fulton
Michigan State University

To determine the pathologic characteristics of isolates from recent outbreaks of infectious laryngotracheitis (ILT) in Michigan and to determine if the virus increased in its ability to cause disease with spread from farm to farm, 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 6 days after challenge 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.

Other authors: Roger Maes, Cunqin Han, Michigan State Univ.

Haemoproteus Iophortyz Infection in Bobwhite Quail

Carol Cardona, Bill Johnson, and Arthur Ihejirika
University of California, Davis

Captive bobwhite quail from a ranch in Northern California have experienced mortality reaching 20-30% during the summer months for the past 14 years. The mortality begins at approximately 5 weeks of age and returns to baseline by 9-10 weeks of age. Clinically affected birds are reluctant to move and are depressed. Blood smears taken from affected birds had intra-erythrocytic gamtocytes, which were identified as Haemoproteus lophortyx. Schizonts were observed in many organs of the body including spleen, lung, skeletal muscle, and liver. The schizonts in skeletal muscle were very large and could be seen grossly as hemorrhagic or white streaks.

New, Reliable and Non-radioactive Colorimetric Assay to Monitor Lymphocyte Proliferation During *Eimeria tenella* Infection.

Hyun S. Lillehoj, Tadashi Miyamoto, and Wongi Min
Parasite Biology, Epidemiology, and Systematics Laboratory,
Animal & Natural Resources Institute, USDA-ARS, Beltsville, MD

The application of a tetrazolium salt, WST-8 to lymphocyte proliferation assay in chicken system was evaluated. In general, the sensitivity of the WST-8 assay was significantly higher than that of the MTT assay. The WST-8 assay was fast, highly reproducible, and provided a good indication of mitogen-induced proliferation of spleen cells induced by Con A. Additionally, the measurement of IL-2 production using WST-8 was highly reproducible. WST-8 assay is safe, fast, simple, and more reproducible and sensitive than MTT assay. This study demonstrates the effectiveness of WST-8 assay to assess cell-mediated immune response in chickens.

Coccidiosis Control and Immunity Development Using Clinacox (Diclazuril) or Salinomycin in Extended Withdrawal Programs

Greg F. Mathis
Southern Poultry Research, Inc.

A 56-day broiler floor pen study was conducted to compare the effects of 1 ppm Clinacox withdrawal at day 28, 35, or 42 and 66 ppm salinomycin withdrawal at day 28 on bird performance under conditions simulating commercial production. The effect these anticoccidial medication programs have on the development of natural immunity to *Eimeria tenella* was determined. Weekly oocyst counts demonstrated that oocysts shedding in the nonmedicated and Salinomycin treatments increased to peaks at 3 to 5 weeks. The highest counts in the Clinacox treatments were on Day 14, while the birds were still on Salinomycin + Roxarsone. Immediately after introduction of Clinacox in the grower diet, oocyst counts dramatically deceased and remained low even after Clinacox withdrawal. The performance results demonstrated that in anticoccidial shuttle programs involving withdrawal as early as 28 days, some slight performance loss might be expected. Due to the

greater efficacy of Clinacox, the extent of the performance loss was substantially less in the Clinacox Day 28 treatment in comparison with the Salinomycin Day 28 treatment. Birds were coccidial challenged on Day 49. Based on bird performance and cecal lesion scores, birds in the Clinacox Day 35 and 42 treatments had little immunity to the challenge infection, birds in the Clinacox Day 28 treatment had a partial immunity. The highest levels of immunity were observed in the nonmedicated and Salinomycin Day 28 treatment. These results show that Clinacox does dramatically and effectively control coccidiosis but in doing so slows development of acquired coccidial immunity.

Productive Performance of Broilers Protected with a Nonattenuated Eimeria Vaccine

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A non attenuated Eimeria commercial vaccine, containing four Eimeria species, was tested in broilers in order to elucidate its effect on the productive parameters. Thirty thousands chickens of one-day old with uniform body weight and from a single breeder flock were divided in two groups of 15,000 each. One group was vaccinated by coarse spray at one-day old in hatchery. The remaining group did not receive vaccine, and was feeding with an anticoccidial supplement. In both groups were recorder weekly for body weight, food consumption, feed conversion, and mortality rate. The level of coccidial infection was determined by scoring gross and microscopic lesions at 14, 21, 28, 35 and 42 days old. Final vaccination performance was judged in 1000 broilers of each group by recording body weight, skin pigmentation and flock uniformity at 46 days old. The findings of the present study have been analyzed to determine the significant effect of the tested vaccine.

The Performance of Maxiban® (Narasin and Nicarbazin) and Nicarbazin in Broiler Starter Rations With and Without Bacitracin Methylene Disalicylate

Bret Rings and Robert L. Cochrane
Elanco Animal Health

A series of three trials were conducted to compare the performance of broilers during the starter period when fed rations containing Maxiban® (narasin and nicarbazin) and nicarbazin in broiler starter rations with and without bacitracin methylene disalicylate. The three trials were conducted in Arkansas using 10 replications per trial with 100 sexed (50 male:50 female) birds per pen for a total of 3,000 birds per treatment. A mixed coccidial challenge containing *Eimeria acervulina, E. maxima*, and *E. tenella* was administered at 14 days. Birds fed starter rations containing Maxiban® (narasin and nicarbazin) statistically outperformed ($p<0.05$) nicarbazin for body weights, average daily gain, and unadjusted feed conversion during the starter period (0-16 days).

An Evaluation of the Association Between Gut pH, Feed Passage, Coccidial Lesions, and Clostridium Perfringens in Broiler Chickens submitted to the Salisbury Animal Diagnostic Laboratory

Fidelis N. Hegngi, Nathaniel L. Tablante, Pierre Brunet, and Pete Warner
Virginia-Maryland Regional College of Veterinary Medicine,
Maryland Department of Agriculture, Salisbury Animal Health Laboratory

Objective - To evaluate the association between gut pH, feed passage, coccidial lesions and *Clostridium perfringens* in broiler chickens submitted to the Salisbury Animal Health Diagnostic Laboratory.

Design - Retrospective study.

Animals - Broiler chickens submitted to the Salisbury Animal Health Diagnostic Laboratory.

Procedure - Broiler chicken routinely submitted to the Salisbury Animal Health Diagnostic Laboratory from January, 1999 - January, 2001 were evaluated for the potential association between feed passage, level of coccidia, and gut pH., with the level of establishment, distribution, and growth of *C. perfringens* in the gastrointestinal tract and the subsequent development of clinical necrotic enteritis.

Results - Gross and microscopic coccidia findings were consistent with increased level of *C. perfringens* in the gut. If a section of gut had increased level of coccidia, presence of feed passage, and an average pH of 5.5, then the establishment, colonization, and frequency of recovery of *C. perfringens* increased. These risk factors favored the proliferation of *C. perfringens* in the intestinal tract and were essential for the subsequent development of clinical necrotic enteritis.

Conclusions - Clostridia is part of the normal microflora in especially the ceca of a chicken. This study confirms that some risk factors as feed passage, pH, and coccidia lesions appear to facilitate the migration, distribution, establishment, and growth of *C. perfringens* in the upper gastrointestinal tract.

The Effects of Chemical Litter Treatment on Clostridia perfringens Induced Necrotic Enteritis in Broiler Chickens: A Pilot Study Verifying A Laboratory Model

M.P. Martin, P.S. Wakenell, R.J. Chick, C. O'Brien, M.C. Bland, D.B. Link, and E. Stroment

Necrotic enteritis is a problem in commercial broilers, which can cause significant mortality and economic losses. There are still large gaps in our understanding of the complete pathogenesis and reduction of the disease. Many agents have been associated with the clinical signs of necrotic enteritis including Clostridia perfringens, coccidia, and Infectious Bursal Disease Virus. Predisposing factors include host animal genetics, agent factor such as high bacterial/coccidial load, and environmental factors such as litter and feed types. Litter acidifiers have been used to show significant reduction in certain bacterial pathogens in poultry environments. A research model that could evaluate the effects of commercial chemical litter treatments on the reduction of Clostridia perfringens and subsequently necrotic enteritis would aid in the understanding of the pathogenesis and reduction of necrotic enteritis.

A pilot study was designed to simulate field exposure to Clostridia perfringens through challenge with contaminated litter. By contaminating rice hulls with a high dose of Clostridia perfringens and a coccidia vaccine, we hope to reproduce the clinical signs of necrotic enteritis in a laboratory environment. Success of the pilot study would establish our model for creating a field-like challenge of necrotic enteritis and will facilitate continued research on the effects of chemical poultry litter treatment to reduce necrotic enteritis in broilers.

Project Description - We obtained 1-day-old broiler chickens from a commercial broiler source and placed 40 birds per group in two groups for positive and negative controls. Litter in the positive control room was inoculated prior to placement of the chicks with a cocktail of four C. perfringens Type A isolates from California Animal Health and Food Safety Laboratory in Fresno from broiler field cases as well as Coccivax-B at 2-4 times the labeled dose per bird. Room size was adjusted to approximate commercial industry standards (approximately 0.80 square feet/bird). Litter consisted of rice hulls and feed obtained from a commercial broiler source to simulate commonly used materials in the broiler industry.

Birds were terminated at 5 weeks of age. Cultures were taken from 3 sites per bird. Cultures were incubated in anaerobic conditions and suspect colonies were evaluated by gram stain and further speciation techniques. Birds that died prior to termination of the project were necropsied for evaluation of gross lesions and cultured with 24 hours.

Poster Program

Poster 1

Relative Importance of Biosecurity Measures: A Delphi Study

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North Carolina State University College of Veterinary Medicine

Virtually no study with hard data has been published on biosecurity measures in poultry. As part of a broader study on biosecurity, a Delphi study was designed to harvest the opinions of poultry health experts on the relative importance of biosecurity measures on the disease status of commercial poultry flocks. The Delphi study design was first developed by the US Air Force in order to collect data on expert opinions on topics where data is not readily available. We are using this approach to quantitatively assess the opinion of 45 poultry health professionals on biosecurity measures and compliance. This study is being conducted at the time of submitting this abstract. Biosecurity measures being considered include people related factors such as access restrictions, footwear, clothing, footbath use, hand sanitation, visit pattern (younger to older birds), employee incentives and exposure to educational material on infectious diseases and biosecurity. Farm level factors include management procedures such as all-in all-out production, the presence of a gate at the entrance of the farm, requirements for vehicles, parking area, wash station, dead bird disposal method, pest control, downtime between flocks, etc. Regional level factors comprise distance between farms, routing of vehicles, regional disposal of used litter, zone raising, and coordination of activities between poultry companies

Poster 2

Biological and Molecular Characterization of a Novel Quail Herpesvirus

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Marek's disease virus (MDV), a herpesvirus and the etiologic agent of Marek's disease (MD), causes severe lymphoproliferative disease in chickens and to a lesser degree in quail and turkeys. The objective of this experiment is to characterize an MDV-like herpesvirus isolated from QT35 cells (Yamaguchi et al, 2000), henceforth referred to as QMDV. Thirty specific-pathogen-free (SPF) chickens were inoculated with QMDV in two separate trials. In the second trial, 30 separate SPF chickens were inoculated with the RB-1B strain of MDV to compare the biological properties and pathogenicity of the QMDV isolate to a known MDV strain. In the first trial, 3 of 30 chickens inoculated with QMDV developed MDV-like tumors. Preliminary results of the second trial indicate that QMDV is indeed capable of causing tumors but that it is less pathogenic than RB-1B. Final necropsy results and histopathological data will be presented.

Poster 3

The Effects of *Riemerella (Pasteurella) anatipestifer* on Pekin Ducks

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Veterinary Preventive Medicine, Ohio State University (Sarver, Morishita, Saif)
Maple Leaf Farms (Nersessian)

Objective - The purpose of this study was to investigate the dosages and potential routes of infection of the bacterial agent, *Riemerella (Pasteurella) anatipestifer* (RA) in Pekin ducks (*Anas platyrhynchos*).

Design - Ducks were given various dosages of RA at different sites and observed for seven days post-inoculation (DPI). Necropsy was performed either on the day birds died, or after seven DPI. Tissues from all birds were collected for bacterial culture and histopathologic examination.

Animals - One hundred and forty-seven, 14-day-old Pekin ducks were obtained from a commercial hatchery and housed in isolated rooms with wood shavings as the flooring substrate. The birds were given a commercial duck feed and water *ad lib*.

Procedure - Twenty-one day old ducks were inoculated by oral, nasal, subcutaneous or intravenous routes with 10^2, 10^4 or 10^6 colony forming units (CFU) per ml of *Riemerella anatipestifer* serotype 2 and observed for seven days.

Results - Mortality was highest in the groups inoculated subcutaneously and intravenously, especially in the 10^4 and 10^6 dose groups. Bacterial isolation rates were greatest from the livers and hearts. Fibrinous pericarditis, perihepatitis and meningitis were seen grossly and microscopically. Lymphoid depletion and/or necrosis were also noted in spleens and bursae.

Conclusions - Study findings indicate that: 1) this RA strain is pathogenic in a dose-dependent manner; 2) the route of exposure is important for clinical expression; and 3) the severity of the lesions may be associated with lymphoid changes.

Poster 4

Experimental Inoculation of Pigeons (Columba livia) with *Mycobacterium bovis*

Laura Zwick, Scott Fitzgerald, and Willie Reed
Michigan State University

Eighteen pigeons (Columba livia) were divided into three groups: a control group, and two groups which were inoculated either orally or intratracheally with a Mycoplasma bovis strain of white-tailed deer origin. Two birds from each group were euthanized at days 30, 60, and 90 post-inoculation, and representative samples of major organs were retained for histologic evaluation and mycobacterial culture. Additionally, mycobacterial cultures of feces were performed at mutiple intervals. The purpose of this study was to determine the susceptibility of pigeons to Mycoplasma bovis, and to develop a risk analysis for thier role in the spread and transmission of this organism.

Poster 5

Avulsion of the Common Retinaculum in Meat Turkeys

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California Animal Health & Food Safety Laboratory System, University of California, Davis (Crespo),
Zacky Farms (Hall), Nicholas Turkey Breeding Farms (Ghazikhanian)

Objective - Investigation in the cause of increased leg condemnations in meat turkeys.

Design - Clinical report.

Animals - Male and female turkeys, between 10 and 19 weeks of age, from different flocks.

Procedure - Health and vaccination histories in the flocks were reviewed. Necropsy, histologic evaluation, bacteria and virus isolations were performed. Mineral content of the bone and tendons were measured.

Results - Affected legs had hematomas around and above the tibiotarsus-tarsometatarsus (hock) joint. Avulsion of the common retinaculum from the external condyle was observed, when the skin and muscular fascia were removed. Interestingly, birds with avulsion of this fibrinous bridge were not lame. The common retinaculum is a band of fibrous tissue that bridges over the tendon of the tibialis cranialis muscle. Body weights were normal, no infectious agents were detected, and mineral content of bones and tendons were within normal limits.

Conclusions - Avulsion of the retinaculum might have not been diagnosed in the past because the birds are not lame and the lesion is tightly covered by the skin and muscular fascia.

Poster 6

Antigenic Relationship of Turkey Coronavirus Isolates in the U.S.

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The objective of the present study was to determine the antigenic relationship of turkey coronavirus isolates in the U.S. Turkey coronavirus isolates from different geographic locations in the U.S. were obtained and propagated in the turkey embryos. Infected turkey embryo intestines were reacted with polyclonal or monoclonal antibody to turkey coronavirus, infectious bronchitis virus, bovine coronavirus, transmissible gastroenteritis, reovirus, rotavirus, adenovirus, or enterovirus in immunofluorescent antibody assays. Positive fluorescent staining was seen with antibodies to turkey coronavirus and infectious bronchitis virus, but not with antibodies to other viruses. The results indicate that turkey coronavirus isolates from different geographic locations in the U.S. were antigenically closely related to each other and to infectious bronchitis virus.

Poster 7

Development and Validation of a Competitive Enzyme-linked Immunosorbent Assay for Detection of Turkey Coronavirus-specific Antibodies

James S. Guy, Lynda G. Smith, and Jamie J. Breslin
North Carolina State University

Objective - The objective of this study was to develop and validate a competitive enzyme-linked immunosorbent assay (cELISA) for detection of turkey coronavirus (TCV)-specific antibodies.

Design - Assay development and validation.

Procedure - Turkey coronavirus nucleocapsid (N) protein was expressed using a recombinant baculovirus (*Autographa californica* nuclear polyhedrosis virus); this recombinant protein was utilized as antigen in the cELISA. Monoclonal antibody (Mab) specific for TCV N protein was produced; this antibody was used as competitor antibody in the cELISA. Sensitivity and specificity of the cELISA for detection of TCV antibodies was determined by comparison with the indirect immunofluorescent antibody test (IFAT) using 1300 reference, experimental and field sera. Sera with discordant cELISA and IFAT results were further evaluated by western immunoblot analyses.

Results - The cELISA detected antibodies specific for TCV and infectious bronchitis virus, a closely related coronavirus, but did not detect antibodies specific for other avian viruses. A high degree of correlation was observed between the cELISA and IFAT; western immunoblot analyses of discordant sera provided additional evidence of cELISA specificity.

Conclusions - The findings indicate that the TCV cELISA is a rapid, sensitive and specific serological test for detection of TCV antibodies in turkeys.

Poster 8

Analysis of the Polyprotein Catalytic Site on Infectious Bursal Disease Virus (IBDV) Protein VP4 by Site-directed Mutagenesis

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Virus protein VP4 of Infectious Bursal Disease Virus (IBDV) is a protease which separates VPX and VP3 of the polyprotein post-translationally. In type I (classic pathogenic) IBDV strains the catalytic site and substrate in which VP4 is active have been demonstrated recently. However, in type II (non-pathogenic) OH-IBDV strain those sites remain unknown. We looked to analyze the protein products of OH-IBDV expressed in vitro during a transcription and translation in rabbit reticulocyte lysates following five different mutations on VP4. We wished to evaluate the importance of serine (S) and aspartic acids (D) on the cleavage site on at VPX and VP4 junction and to analyze the role of the proposed H547, D590 and S653 catalytic triad of the virus chymotrypsin-like serine protease. Our results suggest that the replacement of Serine by Lysine (K) in AXAAS motifs in avibirnavirus type II influences polyprotein (PP) processing, affecting the catalytic capacity of VP4 protease, and

also indicate the presence of an alternative cleavage site. Mutation on D (^{510}TLAADK^{515}) affected the complete PP processing, influencing the cleavage of VPX-VP4, but we have found that independently of the importance of those in LAA, D has an important role as part of the cleavage site and surely participates actively like substrate to the correct cleavage of VP4 protease. Replacement of histidine by proline H547P completely abolished PP processing. Mutation on Aspartic acid (D) in the position 589 induced a partial PP processing when it was replaced by Proline (P) D589P and the introduction of Serine by Proline at the position S652P induced a prominent change in PP processing. All the results permit us to conclude that IBDV VP4 has the ability to act according to structural and topographical changes during translational and post- translational processes, and allow multiple hit sites in order to increase its effectiveness.

Poster 9

Trafficking in the Chicken Lymphoid Tissues of Recombinant Plasmid DNA Expressing the VP2 Capsid Protein Gene of Infectious Bursal Disease Virus

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The mechanisms involved in the uptake, distribution, expression and immunogenicity of plasmid DNA after it is injected parenterally into higher vertebrates remain to be elucidated. Trafficking experiments performed in mice have shown that DNA is taken up, expressed and distributed to the lymph nodes and spleen; however, little is known about the fate of potentially immunogenic DNA molecules after they are injected into chickens. The main objective of this study was to determine whether recombinant plasmid DNA molecules injected intramuscularly into chickens, expressed the gene of interest *in vivo* and could be detected, using the polymerase chain reaction (PCR) technique in primary and secondary lymphoid tissues. The VP2 capsid protein gene of the standard challenge strain of infectious bursal disease virus (IBDV) was cloned into a eukaryotic expression vector and mg amounts of purified DNA prepared by conventional procedures. At three weeks of age, fourteen chickens were injected at two sites in the breast muscle with a total of 500mg of plasmid DNA dissolved in sterile phosphate-buffered saline (PBS). Seven chickens were similarly injected with PBS alone. Samples of muscle, spleen, thymus, bursa of Fabricius and cecal tonsils were collected at various times post injection. After the extraction of total DNA from these tissues, PCR amplification with primers specific for the VP2 gene was used to detect recombinant plasmid DNA. Expression of the VP2 capsid protein was demonstrated in muscle tissue at 12 and 24 hours post injection using a triple sandwich indirect immunoflourescence assay. PCR analyses showed that the recombinant plasmid DNA had been dislocated to lymphoid tissues such as the spleen, thymus and bursa of Fabricius but not to the cecal tonsils, at specific times after its intramuscular injection. No fluorescence or PCR amplification was demonstrated in tissues of sham-inoculated chickens. These results have shown that plasmid DNA injected directly into the breast muscle of chickens is transcribed and translated at the injection site and promptly distributed to primary and secondary lymphoid tissues. Whether these DNA molecules continue expressing the gene of interest as they are transported to the lymphoid tissues remains to be determined.

Poster 10

The Sequence Evidence of Swine Influenza Viruses Infecting Chickens and Turkeys

David Suarez
USDA, Southeast Poultry Research Laboratory

Swine influenza viruses were first isolated from turkeys during an U.S. outbreak in 1980, and other H1N1 isolates have been isolated from poultry on several occasions since then. These outbreaks caused egg production drops and occasionally respiratory disease with mortality. The nucleotide sequence of four different genes from 17 different H1N1 or H1N2 poultry isolates were compared. Swine origin influenza viruses were identified from both chicken and turkey isolates as well as avian origin influenza viruses from a number of different species. A single H1N1 and a H1N2 influenza virus from turkeys had a mixed origin.

Poster 11

Genetic Diversity of Campylobcter jejuni from Broiler Chickens at Processing

Joan Jeffrey, Karen Tonooka, Joan Lazano, and Allison Hunter

The objective of this study was to compare the genotypes of skin and intestinal isolates of *Campylobacter jejuni* (*C. jejuni*) among chickens from different ranches and within a chicken. We used pulsed-field gel electrophoesis of *C. jejuni* DNA following restriction enzyme digest with SmaI and SacII, and analyzed gels using Diversity database software (BioRad, Inc.). The genetic variation observed between isolates from the same chicken, and among ranches will be reported.

Poster 12

Isolation and Characterization of Fluoroquinolone-resistant Campylobacter sp from Poultry Samples

Hashim Ghori and Mohammed Nawaz
Arkansas Livestock and Poultry Commission

Campylobacter sp. has gained importance as a food borne pathogen. Fluoroquinolone antibiotics are used to treat human infections. We have isolated 21 fluoroquinolone resistant campylobacter strains from contaminated chicken liver samples. Morphological, biochemical and fatty acid methyl esters (FAME) analysis indicated that 19 of the 21 isolates were *Campylobacter jejuni* and the two were *C. coli*. All isolates were resistant to multiple antibiotics. These results indicate that several different strains of fluoroquinolone-resistant Campylobacter spp. may be present in contaminated poultry products.

Poster 13

Effects of Maternal Antibodies on ALV-J Infection in Broiler Chickens: Parenteral Injection of Virus

Saad Gharaibeh, Tom Brown, Mary Pantin, and Yongbaek Kim

This experiment was designed to determine the effects of in ovo antibody on virus injected at hatching. Two groups of ALV-J negative embryonated chicken eggs were obtained. At 4 days of incubation eggs were inoculated in the yolk sac as follows. Group 1 was inoculated with neutralizing antiserum against cloned ALV-J (ADOL-7501). Group 2 was inoculated with an equal volume of nonimmune serum. At hatch both groups were challenged with 0.1 ml ($10^{5.5}$ $TCID_{50}$) of cloned ALV-J (ADOL-7501) intraperitoneally. The chicks were raised together until 13 weeks of age. Body weights, tumor formation, viremia status, and antibody status differences between groups will be discussed.

Poster 14

Efficacy of Water Administration of Sodium Acid Sulfate in Reducing Crop Contamination During Feed Withdrawal

J. Allen Byrd and Trisha Marsh Johnson

Water administration of organic acids has been shown to be effective in reducing crop contamination during feed withdrawal. However, this is accompanied by a decrease in water consumption. This study was conducted to determine the efficacy of SAS in reducing Salmonella contamination without negatively affecting water consumption. In a summary of three replicates, 16/40 birds in the 0.250 g/LSAS group were Salmonella positive compared to 24/39 for the control birds and 18/40 for the 0.5% lactic acid group. Water consumption for the SAS group was identical to the controls compared to a 45% decrease in consumption with the lactic acid group.

Poster 15

Salmonella enteritidis Cross Contamination of Table Eggs

Martine Boulianne and Serge Messier
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In view of the upcoming inplemenation of HACCP procedures, we wanted to verify a) if fresh eggs and older eggs may be contaminated when placed in contact with *Salmonella enteritidis* (SE) either in a dry or moist environment, b) if storage temperature and duration might influence SE growth, and c) which part of the egg might be contaminated with SE after such contact. Fresh eggs (still warm) and seven-day-old eggs were placed in 10-6 cfu/ml SE contaminated water or sawdust and then stored at 12°C or 25°C for 24 hours or 25 days (12°C group only). A total of 1728 eggs were tested. Bacterial growth was verified both in the shell and shell membrane and in the albumen. Only the shell and shell membranes tested positive for SE. The number of SE bacteria was influenced by age of the eggs, contaminating medium and storage duration.

Poster 16

Initial Screen for Presence of *Salmonella* in Poultry Environments with Nested - PCR

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Isolation of *Salmonella* generally relies on culture enrichment and subsequent phenotypic screens. We looked into the possibility of using PCR to streamline our current enrichment protocol and as a reference for the delayed enrichment. The unique *Salmonella* virulence gene *invA* was chosen as target for nested-PCR due to its uniform distribution among *Salmonella* serotypes. Nested-PCR increased the sensitivity by 1000-fold and was used to screen primary enrichments for *Salmonella*. A strong positive correlation between PCR and culture was observed. This method will not only validate enrichment procedures but also reduce costs and manpower for surveillance of *Salmonella* in livestock.

Poster 17

Genetic Variability of Reticuloendotheliosis Provirus in the Genome of Fowlpox Virus

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The presence of nearly intact provirus copies of reticuloendotheliosis virus (REV) in the genome of fowlpox virus (FPV) was initially described for a field isolate originating in and a standard vaccine strain previously used in Australia. We have since found that a similar integration event has occurred in viruses isolated earlier and in those associated with natural outbreaks of fowlpox in chickens in the United States. Although the site of integration is identical, these geographically separated FPV strains can be distinguished by the presence of multiple nucleotide substitutions and deletions in the 3' region of the provirus. These differences are reflected by the divergence in the predicted amino acid sequences of the carboxyl terminus of the envelop protein of the integrated REV. Interestingly, a similar lack of conservation in this region exists between two REV strains naturally occurring in the United States. A comparison of the primary structure of their envelop proteins indicates that only one of the REV variants is closely related to the provirus in the genomes of three FPV isolates of United States origin. In contrast, a virus similar to the other REV strain is present in the DNA of the Australian FPV. Thus, REV integration into the FPV genome was probably not a unique event and this interaction between the two viruses probably is advantageous to their continual existence.

Poster 18

Molecular Characterization of Reticuloendotheliosis Virus (REV) Insertions in the Genome of Field and Vaccine strains of Fowl Pox Virus (FPV)

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Department Avian Medicine, University of Georgia (Garcia),
Animal Health Diagnostic Laboratory, Michigan State University (Reed)

Molecular characterization of Australian vaccine and field strains of fowlpox virus (FPV) have shown the presence of a near full length infectious reticuloendotheliosis virus (REV) genome integrated into a vaccine strain of FPV. Fowlpox vaccines contaminated with REV have also been identified in samples from the USA. However, it is not known if the apparent contamination is due to free REV or to REV arising from integrated provirus. The objective of this study is to determine if REV contaminated FPV strains arise from free or genome integrated REV. Polymerase chain reaction (PCR) amplification procedures were developed to screen for REV sequences in one vaccine and four field strains of FPV. Three of the field isolates and the vaccine strain showed positive amplifications using REV primers located at LTR, *env*, *pol*, and *gag* regions, indicating the presence of different sequences of REV provirus in FPV preparations. Using FPV primers, which flank the previously identified LTR REV region within FPV, amplification was obtained from three of five samples. In addition, using REV and FPV primers, located at the REV *env* and at the FPV *PK* gene, three of the five samples tested positive by PCR. The data, indicate that REV sequences are integrated in three FPV samples. Sequence analyses are being conducted to find the exact site and extent of REV integration.

Poster 19

Condor Poxvirus with Biological Differences from Fowlpox Virus

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A pox virus was isolated from the spleen of a natural systemic infection in an Andean condor. Although the virus produced characteristic lesions and cytoplasmic inclusion bodies on the chorioallantoic membrane of developing chicken embryos, it failed to grow in avian cell cultures. Chickens immunized with the virus developed localized pox lesions at the site of inoculation similar to fowlpox virus. However, birds immunized with this virus were not protected against challenge with fowlpox virus.

Poster 20

Characterization of a Possible Avian Macrophage Chemoattractant from an Avian Macrophage EST Library

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Department of Animal and Food Sciences, University of Delaware

Macrophages are important modulators of the immune response. We have constructed an EST library from the LPS-stimulated HD11 avian macrophage cell line. Over 1,200 clones from this library have been sequenced and subjected to BLAST analysis. Eleven percent of the clones represent genes with potential immunological functions. From this EST library a possible chicken homolog to human Endothelial Monocyte Activating Polypeptide-II (EMAP-II) was identified. This protein is predicted to have macrophage chemotactic properties as well as an apoptosis/anti-tumor role. A full-length clone of this gene (chEMAP-II) was attained using 3'-RACE. The gene is 927 nucleotides in length and is predicted to encode a 308 amino acid polypeptide. After proteolytic cleavage the active form of the protein is contained within the C-terminal 166 amino acids. BLAST analysis shows this portion of the predicted polypeptide to be 84% identical to the human EMAP-II protein. chEMAP-II is being expressed in pRSET and the recombinant protein will be evaluated for chemoattractant properties.

Poster 21

Effect of Toe Trimming, Probiotics and Litter Acidification on the Incidence of Cellulitis in Broiler Chickens

Michelle Tessier, Philippe Labelle, Robert Gauthier, and Martine Boulianne
University of Montreal and Jefo Nutrition, Inc., Canada

One day old broiler chickens (n=1,600) were divided into two main groups: toe-trimmed and intact birds. Birds of each group were then allocated to one of four subgroups 1) daily supplementation of fructooligosaccharides, b) probiotics at one day of age, c) litter acidification at day 27, and d) controls. One day 42, each pen litter was contaminated with a mutated nalidixic acid resistance *Escherichia coli* suspension, and on day 49, two days prior to slaughter, a 1,5cm incision on the left side of the abdomen was made on all birds. 24.7% of the broilers showed cellulitis lesions. There was no significant difference between the toe-trimmed and the intact birds, no between treatments. However, cellulitis was positively correlated with the litter humidity level at day 49.

Poster 22

Correlation of Phenotypic and Genotypic Characteristics and Embryo Lethality in Identifying Virulent and Commensal Avian *Escherichia coli*

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Catherine W. Giddings, Shelley M. Horne, and Steven L. Foley
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University of Georgia (Wooley, Gibbs), Department of Veterinary and Microbiological Sciences,
North Dakota State University (Nolan, Giddings, Horne, Foley)

Objective - Determine which phenotypic and/or genotypic laboratory tests correlate with the embryo lethality assay for establishing the virulence status of avian *Escherichia coli* isolates.

Procedure - Ten field isolates of *E. coli* from cases of colibacillosis and ten *E. coli* isolates cultured from the intestinal tracts of normal broiler chickens were assayed for selected phenotypic and genotypic characteristics. These tests included the embryo lethality assay, bacterial resistance to chicken complement, colicin activity, motility, type F1 fimbriae, and the presence of the increased serum survival (*iss*) and the *arsH* genes.

Results - The presence of Colicin-V and the increased serum survival gene (*iss)* are reliable tests that may be used to identify virulent and commensal avian *E. coli.*

Poster 23

C3b Deposition on Avian *Escherichia coli*

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Objective - To improve complement-resistance assays to be used in assessing the virulence of avian *Escherichia coli* isolates since complement resistance appears to be predictive of *E. coli* virulence.

Experimental Design - *E. coli* isolates from healthy and sick birds were studied with three different complement-resistance assays, and the results examined for their abilities to predict virulence.

Procedures - *E. coli* isolates were subjected to three complement-resistance assays including, a viable count method, a flow cytometric method using a commercial staining kit to distinguish live from dead cells, and a flow cytometric assay designed to detect C3b deposition on the bacterial surface. Results of these tests were correlated to those obtained with these same isolates in an embryo lethality test of virulence.

Results and Conclusions - Studies are ongoing but promise to identify a reliable measure of complement resistance that may prove to be good indicator of avian *E. coli* virulence.

Poster 24

Plasmid Location of *iss* in an Avian *Escherichia coli* Isolate

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Objective - We have found that the presence of the increased *s*erum *s*urvival gene (*iss*) in an avian *E. coli* may be a good predictor of that isolate's potential to cause disease. In the present study we have sought the location of *iss* in the genome of an avian *Escherichia coli* isolate since its location may provide insight into *iss*' role in the pathogenesis of avain colibacillosis.

Design - *Iss* has been shown to occur on a ColV plasmid in a human *E. coli* isolate. Therefore, *E. coli* isolates that are implicated in avian colibacillosis and known to contain *iss* were used as donors in conjugation experiments. Transconjugants were then assessed for acquisition of *iss*, virulence, complement resistance, and genes thought to contribute to avian *E. coli* virulence.

Procedure - Briefly, naladixic-acid sensitive donor strains were incubated with naladixic-acid resistant, plasmidless, avirulent, recipient strains under conditions known to promote conjugation. Transconjugants were selected on agar containing donor- and recipient-inhibitory concentrations of antibiotics. Transconjugants were examined for plasmids containing sequences homologous to *iss* and other potential virulence genes, including *cvaC*, the structural gene for ColV, and *iucA* and *iucC*, genes of the aerobactin operon, using DNA:DNA hybridization techniques. Additionally, transconjugants were examined for their acquisition of virulence and complement resistance using bioassays.

Results and Conclusions - Although this study is ongoing, our results to date indicate that *iss*, in the one isolate examined, occurs on a large transmissible R plasmid. Therefore, further study may reveal that antibiotic pressure is an issue in selection for avian *E. coli* virulence.

Poster 25

Screening a Genomic Library of a Virulent Avian *Escherichia coli* Isolate for *iss*

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Objective - We have found that the presence of *iss* DNA sequences is strongly correlated with *E. coli* implicated in avian colibacillosis. Our objective is to determine the location of *iss* in the genome

Design - A genomic library of a virulent, *iss*-containing *E. coli* isolate was generated by cloning 40-kb inserts into a cosmid vector. We will then screen the library for *iss,* and sequence clones containing *iss.*

Sample Population - We are using a virulent, complement-resistant avian *E. coli* strain.

Procedure - We have produced a cosmid library of a virulent, complement-resistant avian *E. coli* known to contain *iss.* Total DNA was isolated from the virulent *iss*$^{+}$, complement-resistant avian *E. coli* by the modified Marmur protocol. The DNA was briefly digested with *Sau 3A* and size fractionated in a 20% to 60% sucrose gradient to isolate DNA fragments of about 40 kb. The 40 kb DNA fragments were ligated into the cosmid vector Supercos (Statagene) and packaged into lambda. The library was transfected into a recipient *E.* coli K12 strain for screening. The colonies obtained were screened for the presence of the *iss* gene by Southern analysis.

Results and Conclusions - The library consists of 600 clones, each containing a genomic DNA insert of about 40 kb. A cosmid clone containing *iss* has been identified by DNA:DNA hybridization techniques. Sequencing of this clone is ongoing.

Poster 26

Prevalence of Enteropathogenic *Escherichia coli* (EPEC) in Naturally-occurring Cases of Poult Enteritis Mortality Syndrome (PEMS)

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Objective - To gain a better understanding of the prevalence of enteropathogenic *Escherichia coli* (EPEC) in PEMS-affected turkeys.

Design - Retrospective study

Sample populations - Formalin-fixed intestinal tissues and frozen (-75 C) fecal materials from 12-PEMS-affected flocks.

Procedure - Formalin-fixed intestinal tissues were examined for light and electron microscopic lesions consistent with EPEC. *E. coli* were recovered from frozen feces and characterized based on a epithelial cell attachment assay, and presence of genes encoding *E. coli* attaching/effacing (eae) gene, shiga-like toxin 1 (SLT 1), SLT 2, and bundle forming pili (Bfp A) using polymerase chain reaction procedures. EPEC were examined for pathogenicity by experimental inoculation of young turkeys.

Results - Lesions consistent with EPEC (multifocal epithelial degeneration associated with attached bacteria, effacement of microvilli) were identified in intestines of turkey from $^{7}/_{12}$ PEMS-affected flocks. Histopathologic lesions consistent with EPEC were not identified in intestines collected from 6 turkey flocks with enteric disease other than PEMS. *E. coli* isolates from $^{5}/_{12}$ flocks were identified as EPEC based on presence of eae gene and absence of SLT 1 and SLT 2. EPEC isolates did not process bfp A gene.

Conclusions - These findings provide additional evidence suggesting a possible role for EPEC in the pathogenesis of PEMS.

Abstract - Enteropathogenic Escherichia coli (EPEC) previously were identified in poult enteritis-mortality syndrome-affected turkeys from a single grow-out complex, and associated as a cause of this disease. To gain a better understanding of the prevalence of EPEC in PEMS-affected turkeys, a retrospective study was done to examine archived formalin-fixed intestinal tissues for light and electron microscopic lesions consistent with EPEC. Additionally, frozen (-75 C) fecal material were examined for presence of EPEC. E. coli recovered from frozen feces were characterized based on a epithelial cell attachment assay, and presence of genes encoding E. coli attaching/effacing (eae) gene, shiga-like toxin 1 (SLT1), Stx2, and bundle forming pili (BfpA) using polymerase chain reaction procedures. EPEC were examined for pathogenicity by experimental inoculation of young turkeys.

Lesions consistent with EPEC (multifocal epithelial degeneration associated with attached bacteria, effacement of microvilli) were identified in intestines of turkeys from $^{7}/_{12}$ PEMS-affected flocks. Histopathologic lesions consistent with EPEC were not identified in intestines collected from 6 turkey flocks with enteric disease other than PEMS. E. coli isolates from $^{5}/_{12}$ flocks were identified as EPEC based on presence of eae genes and absence of Stx 1 and Stx 2; EPEC isolates did not possess bfpA gene. These findings provide additional evidence suggesting a possible role for EPEC in the pathogenesis of PEMS.

Poster 27

Development of Methods for Detection of Envelope Glycoprotein of Subgroup J Avian Leukosis Virus

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Subgroup J Avian leukosis virus (ALV-J) induces high incidence of myelocytomatosis in meat-type chickens. Successful control of ALV-J infection in breeder flocks is viral eradication program, which is based the sensitivity and specificity of methods used for detection of either antigens or antibodies to the virus. The gp85 envelope glycoprotein of starin ADOL-Hc1 of ALV-J was cloned into baculovirus and pET expression system for abundant expression of the protein. The expressed protein was purified and used to immunize rabbit to generate high titer antiserum to ALV-J. Using either monoclonal antibodies or rabbit anti-gp85 antibody, we detected gp85 antigens in infected chickens by indirect immunofluorescence test. Additional methods for detection of ALV-J antigens such as enzyme-linked immunoabsorbent assay or dot blot are being developed.

Poster 28

Using Ultrasound to Predict Lean Tissue Mass in Broiler Breeder Females

Dr. Sallee J. Dixson, Oklahoma State University
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Dr. Kenneth C. Powell, Monsanto Animal Nutrition

Objective - To determine if ultrasound (U/S) can serve as a non-invasive predictor of lean tissue mass (LTM) in broiler breeder females (BBF).

Design - Repeated measures CRD

Animals - 104 BBF (Cobb) (ages 5 to 50 weeks at 5-week intervals)

Procedure - Using an U/S scanning system (provided by Products Group International, Inc.) with a 10 MHz probe, three breast measurements made just cranial to the keel hook included: ventral-dorsal thickness of (1) the superficial pectoralis muscle (SPm) and (2) the supracoracoideus muscle (SCCm), and (3) medial-lateral width of the SCCm. Both SCCm areas were calculated then totaled for a combined SCCm (combSSCm) area for each bird. Ultrasound measurements were correlated to total LTM as determined with a Hologic 1000W dual-energy X-ray absorptiometry unit (DEXA).

Results - Each U/S measurement (6/bird) and the three calculated SCCm areas were correlated with the DEXA determined total LTM (g). All correlations were > 0.90 except for left and right SPm correlations, which were 0.690 and 0.741 respectively. All p-values were < 0.0001. The U/S measurement most highly correlated to total LTM was the combSCCm area (R=0.965). Regression analysis (SAS ver. 6.12), with total LTM regressed on combSCCm area, yielded an equation for estimating LTM based on U/S measurements of the SCCm.

Lean tissue mass (g) = 258.14 + 2.30(combSCCm area)
R2 = 0.93 and $p < 0.0001$

Conclusions - A regression equation, based on U/S measurements of the SCCm, may be used to predict total lean tissue mass in broiler breeder females ages 5 to 50 weeks.

Poster 29

Feline Aminopeptidase N as a Receptor for Infectious Bronchitis Virus

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Feline aminopeptidase N (fAPN) has been shown to serve as a receptor for feline, canine, porcine and human coronaviruses. Our objective was to determine if fAPN can serve as a receptor for IBV. Feline kidney cells and hamster kidney fibroblasts were infected with Arkansas serotype of IBV and analyzed by indirect fluorescent assay, confocal microscopy and titration in SPF embryonated eggs. The results showed that the feline cells were permissive to IBV but the hamster cells were not. The hamster cells became permissive to IBV after transfection with a fAPN cDNA.

Poster 30

Glycosylation Analysis of the IBV Spike Glycoprotein

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Glycosylation is thought to play a role in the formation of conformationally-dependent virus-neutralizing epitopes on the IBV spike glycoprotein; however, there has been no direct evidence of this to date. In this study, we examined the effect of glycosylation on the binding ability of a neutralizing monoclonal antibody to a cloned spike glycoprotein. The resulting glycoprotein will be analyzed using the neutralizing monoclonal antibody in indirect immunofluorescence and Western blotting, along with glycosylation detection, and compared to the original spike protein.

Poster 31

Synthesis and Analysis of Hammerhead Ribozymes Targeted to Infectious Bronchitis Virus Subgenomic mRNA Coding for the Nucleocapsid Protein

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Hammerhead ribozymes are catalytic RNA molecules that specifically cleave a target RNA molecule. To our knowledge, there are no reports of Infectious Bronchitis Virus (IBV) specific ribozymes in the literature. Herein, we report the synthesis and analysis of hammerhead ribozymes targeted to IBV subgenomic mRNA coding for the nucleocapsid protein. IBV specific hammerhead ribozymes can be useful laboratory tools and have future therapeutic/prophylactic applications.

Poster 32

Use of a Live Coccidial Oocyst Vaccine to Change a Salinomycin-Resistant Field *Eimeria maxima* Population

Linnea Newman, Harry Danforth, Steve Rone, and John McCarty

A US broiler integrator used a live coccidial vaccine to change a field population of salinomycin-resistant Eimeria maxima. Salinomycin sensitivity tests were conducted on field oocyst isolates from a test farm before vaccination, and from the same farm following 3 sequential vaccinated flocks. Reduction of gross E. maxima lesions and in E. maxima detected by intestinal scrapings was documented in post-mortem examinations of vaccinated flocks. The integrator resumed an ionophore program after 3 vaccinated flocks. Litter samples were collected from the next 3 sequential ionophore flocks on the test farm to determine the duration of any field oocyst sensitivity change.

Poster 33

Immunogenetic Assessment of Staphylococcal Tenosynovitis and Osteomyelitis in Broiler Breeder Stock

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Objective - To assess spontaneous Staphylococcal lameness in association with MHC *B* complex genotypes in a pure genetic line of broiler breeders.

Animals and Design - Nine to 21-week-old, chickens from six Line A flocks in a commercial broiler breeder program were observed for ten weeks. Eighty-nine clinically lame and 34 normal, age-matched control birds were evaluated for gross and histopathologic lesions, MHC genotype and bacterial culture. The normal MHC genotype distribution was determined for each flock and chicken infectious anemia virus (CAV) exposure was determined for two flocks.

Procedure - Bacteria were isolated using standard culture techniques and further analyzed via gas chromatography. MHC genotype was determined via hemagglutination with serologic reagents produced by one of the authors (SJE). Randomly selected sera from two flocks were analyzed for antibodies to CAV.

Results - Tenosynovitis, arthritis, and femoral and/or tibiotarsal osteomyelitis were observed grossly or microscopically in 85/89 lame birds. Gas chromatography of 95 obtained bacterial isolates revealed *Staphylococcus aureus* subgroup B (37/95; 38.9%), *S. aureus* subgroup A (7/95; 7.3%), other *Staphylococcus* spp. (29/95; 30.5%) and other bacterial species (22/95; 23.3%). The tested flocks were negative for CAV antibody. Combined flock Chi-square analysis revealed that the MHC genotypes B^{A4}/B^{A4} ($c^2 = 14.54$, $P = 0.0063$) and B^{A12}/B^{A12} ($c^2 = 42.77$; $P = 0.0001$) were over represented in the sample of symptomatic birds compared to the random sample from the same flocks.

Conclusions - The high prevalence of Staphylococcal induced lameness in association with B^{A4}/B^{A4} and B^{A12}/B^{A12} MHC genotypes is suggestive of an MHC *B* complex influence on the expression of bacterial skeletal disease in this line of broiler breeders.

Poster 34

***Staphylococcus aureus* Granulomatous Pneumonia in 6-day-old Turkey Poults**

Alex J. Bermudez and Magalie Boucher
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Staphylococcus aureus infections are a regular occurrence in turkeys and commonly reported disease conditions include omphalitis, cellulitis, septicemia, septic arthritis, synovitis, and osteomyelitis. This report describes a case of *S. aureus* granulomatous pneumonia in a flock of 11,000 male turkey poults that experienced 10% mortality during the first week of production. The female poults from this same hatch, placed on a different farm, experienced a similar mortality pattern.

The gross lesions in this case were highly suggestive of granulomatous pneumonia as is typically seen with pulmonary aspergillosis. Other tissues, including liver, spleen, air sacs, and yolk sac were unremarkable on gross examination. Microscopic examination of the lungs of infected poults revealed multifocal pulmonary granulomas that contained numerous bacterial cocci. Gram stained sections of lung revealed that the bacterial cocci were Gram positive. GMS stained sections of lung were negative for *Aspergillus* spp. fungal mycelia.

The individual lungs of eight poults were examined both microscopically and microbiologically and all eight pneumonic lungs were culture positive for *S. aureus*. Seven of eight liver cultures from these same poults were negative for aerobic bacteria and one liver culture was positive for Enterococcus spp. The gross and microscopic lesions, microbial cultures, and case history suggest a severe *S. aureus* aerosol exposure in the hatchery. Based on the findings of this case report, when the gross lesions of multifocal granulomatous pneumonia are observed in turkey poults, pulmonary staphylococcosis should be included in the differential diagnosis.

Poster 35

Correlation of Tracheal Antigenic Distribution and Levels of Specific Humoral Antibodies in Pneumovirus Infected Broiler Breeders and Their Offspring

Elie Barbour, Faris Jirjis, and Shady Hamadeh

The detection rate of antigenic distribution of pneumovirus in tracheal tissues by immunofluorescence and the quantitation of systemic humoral antibodies specific to pneumovirus were correlated in broiler breeders and their offsprings. The chronological correlations detected at different time intervals before, during, and after the appearance of specific signs and lesions of swollen head syndrome, were determined. The value of the determined correlations in diagnostics will be emphasized.

Poster 36

Reproductive Tract Tumors in Mature Laying Hens

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Objective - To describe the pathology of tumors involving the reproductive tract of mature laying hens.

Design - Reproductive tracts from 1405 four-year-old laying hens were examined for tumors. Birds had been divided into six groups. A control group was given full feed and experienced normal ovulation while the remaining five groups (control and 4 treatment groups given combinations of different possible inhibitors of ovarian carcinoma) were placed on a restricted feeding program that inhibited egg production.

Procedure - Reproductive tracts and internal organs were examined at necropsy. If lesions were discovered the carcass was photographed and representative tumors were removed and placed into 10% formalin. If no lesions were seen, ovary and sections of oviduct were placed into formalin.

Results - The most common tumors were leiomyomas that arose from the muscle layer of the oviduct or within the ventral mesosalpinx. Adenocarcinomas involving the ovary and/or oviduct were the second most common tumor. Histologically, they often had characteristics of oviductal glandular epithelium even if the oviduct did not contain a tumor. Carcinomas were significantly more frequent in full fed birds (33.9%) compared to restricted feed control hens (9.4%). There was a slight further reduction in tumor occurrence among the treated groups (4.9 - 6.8%). Adenomas, sex-cord tumors (Sertoli, granulosa cell), and germ cell tumors occurred infrequently.

Conclusions - Leiomyomas and adenocarcinomas of the ovary and oviduct occurred frequently in mature laying hens. Other tumors were uncommon to rare. Restriction of feed induced anovulation and was associated with a significant decrease in the occurrence of carcinomas.

Poster 37

Ontogeny of Cellular and Humoral Immune Response Parameters in Ducks

Y. Wang, P.R. China, and U. Neumann

Poster 38

Investigations on Immune Cell Populations of the Head-Associated Lymphoid Tissues: Immunohistochemical Aspects, Cell Mediated and Humoral Immune Response in Unvaccinated and Vaccinated Broilers

P. Dumrongsoontornchai and U. Neumann

Poster 39

Drumstick Lesions of Unknown Etiology at a Poultry Slaughtering /Processing Facility

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The Ohio State University

Objective - To determine the etiology of cutaneous bruising found on the distal end of the frozen, packaged drumsticks processed at a poultry slaughtering/processing facility.

Animals - Samples were fresh, processed, whole birds; fresh drumsticks from the processing lines and rejected processed drumsticks; and frozen, packaged drumsticks that had been processed at least four days prior to testing.

Procedure - Fresh and frozen samples were examined for cutaneous bruising on the distal aspects of the drumsticks. Radiology, gross, and histological examinations were performed.

Results - Radiographic evaluation: Samples with cutaneous lesions had no apparent fractures of the bones, and no changes in the soft tissue density of the surrounding area. The bone densities of all samples were within normal limits.

Gross examination - No cutaneous bruising or reddening was found on the fresh whole birds. The processed drumsticks were similar in appearance and few bruised drumsticks were noted. Many of the frozen, packaged samples had reddened areas around the distal end of the drumsticks and the bone marrow was softer than observed in the fresh samples.

Histological examination - Bone and marrow from all samples were within normal limits.

Conclusions - Based on the history and diagnostic tests, infectious and nutritional diseases of the birds prior to slaughter were ruled out. A traumatic cause for the damage was more likely and this damage was limited to the skin and soft tissues surrounding the bone. It is presumed that the shackling system at the plant may be the causative agent of the damage. This will be further studied by histologic examination on the soft tissue of bruised drumsticks as more samples become available.