Poultry Science Association
American Association of Avian Pathologists

Abstracts

July 16–19, 2011
St. Louis, Missouri
America's Center

The Gateway to the Future of Poultry Research

Joint meeting of the Poultry Science Association
and the American Association of Avian Pathologists

Poultry Science Volume 90, E-Supplement 1

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2011 Annual Meeting

July 16-19, 2011 St. Louis, MO

America's Center Convention Complex

Saturday, July 16, 2011 7:30AM - 12:30PM
AAAP and PSA Joint Symposium
A Crystal Ball Look into the Future of...

The symposium is designed to take a look into the future of poultry production in the next 5 to 10 years. Topics will include genetics, nutrition, hatchery, immune modulation, and coccidiosis. The session will be capped by this year's keynote speaker addressing the issue of the Future of Antibiotics in Animal Agriculture.

Saturday, July 16, 2011 1:00PM - 5:00PM
AAAP Committee Meetings
Hyatt Regency Hotel

*Saturday, July 16, 2011 7:00PM - 8:30PM
AAAP/PSA Opening Session & Reception
Hyatt Regency Hotel, Grand Ballroom & Grand Foyer

Sunday- Tuesday, July 17-19, 2011 8:00AM - 5:30PM
AAAP/PSA Scientific Program
America's Center Convention Center Complex

Sunday, July 17, 2011 12:15PM - 2:45PM
AAAP Awards Luncheon
Renaissance Grand Hotel, Crystal Ballroom

*Sunday, July 17, 2011 7:00PM - 8:00PM
AAAP/PSA Ice Cream Social
Hyatt Regency Hotel, Gateway East

Monday, July 18, 2011 1:15PM - 2:15PM
The joint AAAP Lasher-Eckroade History Lecture and the WPSA Lecture, Dr. Greg Mathis
The History of the Poultry Industry: Scientific Breakthroughs
America's Center Convention Center Complex

Monday, July 18, 2011 2:15PM - 4:00PM
AAAP Business Meeting
America's Center Convention Center Complex, Room 222

*Monday, July 18, 2011 4:00PM - 5:30PM
AAAP/PSA Wine and Cheese Reception
America's Center Convention Center Complex- Poster Rooms

AAAP Hotel Headquarters
Hyatt Regency
St. Louis at The Arch
315 Chestnut Street
St. Louis, Missouri, USA 63102

Program information available at
www.aaap.info/2011Meeting

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The American Association of Avian Pathologists strives to prevent and eliminate the suffering and loss of poultry due to disease, to assure a safe poultry- associated food supply and to develop and use cutting edge, science based techniques to meet these goals through education of current and future scientists.

*** New for AAAP Members**

AAAP MEETING AND EVENTS SCHEDULE

Annual Meeting 2011 St. Louis, MO

Name of Group	Meeting Date	Beg. Time	End Time	Room Name
AAAP EVENTS				
AAAP New Member Meet and Greet	Saturday, July 16	7:00am	7:30am	AAAP Booth Convention Center
AAAP & PSA Joint Symposium	Saturday, July 16	7:30am	12:30pm	Convention Center
Opening Session AAAP and PSA	Saturday, July 16	6:00pm	7:00pm	Grand Ballroom, Hyatt Hotel
Opening Reception AAAP and PSA	Saturday, July 16	7:00pm	8:30pm	Grand Foyer, Hyatt Hotel
AAAP/PSA Scientific Sessions	July 17-19	8:00am	5:00pm	Convention Center
AAAP Awards Luncheon	Sunday, July 17	12:15pm	2:45pm	Crystal Ballroom Renaissance Hotel
AAAP and PSA Ice Cream Social	Sunday, July 17	7:00pm	8:00pm	Hyatt Regency Hotel
AAAP Past Presidents Luncheon	Monday, July 18	11:30am	1:00pm	Renaissance Hotel -Benton Room
Lasher-Eckroade/WPSA History Lecture	Monday, July 18	1:15pm	2:15pm	Convention Center
AAAP Business Meeting	Monday, July 18	2:00pm	4:00pm	Convention Center
AAAP/PSA Wine and Cheese Reception	Monday, July 18	4:00pm	5:30pm	Poster Room Convention Center
AAAP Board of Directors Meeting				
AAAP Board of Directors Meeting	Thursday, July 14	7:00am	5:00pm	Sterling 1
AAAP Board of Directors Meeting	Friday, July 15	7:00am	5:00pm	Sterling 1
AAAP Board of Directors Meeting	Tuesday, July 19	7:00am	12:00pm	Sterling 1
AAAP Committees and Interest Group Meetings				
Histopathology/Case Report Interest Group	Friday, July 15	1:00pm	4:00pm	Regency E
Awards Committee	Saturday, July 16	1:00pm	2:00pm	Sterling 1
Drugs and Therapeutics Committee	Saturday, July 16	1:00pm	2:00pm	Sterling 5
Electronic Information Committee	Saturday, July 16	1:00pm	2:00pm	Sterling 7
Food Safety Committee	Saturday, July 16	1:00pm	2:00pm	Sterling 8
Avian Diseases Manual Editorial Board	Saturday, July 16	1:00pm	2:00pm	Sterling 9
Diseases of Poultry Editorial Board	Saturday, July 16	1:00pm	2:00pm	Mills 9
Membership Committee	Saturday, July 16	1:00pm	2:00pm	Mills 6
Biologics Committee	Saturday, July 16	2:00pm	3:00pm	Mills 2
Diseases of Public Health Significance Committee	Saturday, July 16	2:00pm	3:00pm	Mills 1
Isolation, Identification Manual Editorial Board	Saturday, July 16	2:00pm	3:00pm	Mills 4
Preceptorship Committee	Saturday, July 16	2:00pm	3:00pm	Mills 5
Tumor Virus Committee	Saturday, July 16	2:00pm	3:30pm	Mills 7
Animal Welfare Committee	Saturday, July 16	2:00pm	4:00pm	Mills 3
Education Committee	Saturday, July 16	3:00pm	4:00pm	Mills 8
Emergency Disease Management Committee	Saturday, July 16	3:00pm	4:00pm	Sterling 5
Research Priorities Committee	Saturday, July 16	3:00pm	4:00pm	Sterling 4
Respiratory Diseases Committee	Saturday, July 16	3:00pm	5:00pm	Regency B
Enteric Diseases Committee	Saturday, July 16	4:00pm	5:00pm	Park View
Epidemiology Committee	Saturday, July 16	4:00pm	5:00pm	Mills 1
History Committee	Saturday, July 16	4:00pm	5:00pm	Mills 9
TIME Committee	Saturday, July 16	4:00pm	5:00pm	Sterling 7
Legislative Advisory Committee	Sunday, July 17	6:30am	7:30am	Sterling 8
Avian Diseases Editorial Board	Sunday, July 17	7:00am	8:00am	Grand B
AAAP Associated Groups Meetings (Attendance by Invitation Only)				
Association of Veterinarians in Broiler Production	Friday, July 15	7:00am	5:00pm	Park View
Association of Veterinarians in Turkey Production	Friday, July 15	10:30am	5:00pm	Gateway West
Georgia MAM Alumni Breakfast	Saturday, July 16	6:30am	7:30am	Park View
Association of Primary Poultry Breeder Veterinarians	Saturday, July 16	11:30am	1:30pm	Sterling 2
University of Minnesota CAHFS	Saturday, July 16	5:00pm	6:00pm	Regency A
Association of Veterinarians in Egg Production	Sunday, July 17	6:00am	8:00am	Grand A
NC State University Poultry Health Management	Monday, July 18	7:30pm	11:00pm	Grand E
ACPV Meetings				
ACPV Exam	Friday, July 15	7:00am	8:00pm	Grand A & B
ACPV Board of Governors Meeting	Sunday, July 17	7:00am	11:00am	Mills 6
ACPV Reception/Annual Meeting	Monday, July 18	7:00am	9:00am	Grand FG

Avian Diseases Digest

Begun in 2006, Avian Diseases Digest was developed with the non-scientist avian professional in mind, but the busy avian scientist will find it a helpful tool as well. After reading an article in Avian Diseases Digest, the reader will understand what is being published and how the findings relate to him or her as poultry professionals. Avian Diseases Digest functions as a quick over-view of the latest scientific research. It does not take away the need to read Avian Diseases where all the technical details on each article are available. Published four times a year and available online in English and Spanish for $45 a year.

Available at www.aaap.info/page/ADDigest

11031

The Toxicity Effects of Feeding 1-Alpha Hydroxy D3 to Broiler Breeding Hens

Bret Rings, Fred Hoerr, Scott Gustin, John Halley
Veterinarian Responsible For Company Production And Health

One-alpha hydroxy D3 was utilized for replacing a previous source of vitamin D3 in a broiler breeder ration at a 0.5 pound/ton inclusion. Broiler breeders fed this product at this level had clinical effects of flushing, increased urate production, drops in egg production and increased mortality. Necropsy of affected mortality revealed pale kidneys, urolithisis and visceral gout to various extremes. Clinically affected birds were moderately depressed with excessive fecal and urate staining at the feathers surrounding the vent. Culls eggs were significantly increased due to increased fecal staining and the high numbers of floor eggs laid in extremely wet litter. Histopathology indicated kidneys had subacute to chronic moderate nephrosis with basophilic granular tubular casts, predominantly involving segments of the nephron in the medullary cone. Formation was associated with effacement and loss of tubular epithelium, especially in tubules that had the lumens occluded by the casts. There was mild interstitial cellular inflammation of lymphocytes and mononuclear cells observed. The histopathologist commented that such findings were similar to vitamin D or calcium toxicity. Upon removal of the 1-alpha hydroxy D3 and return to the previous source of vitamin D3 flushing ceased and egg production returned but mortality remained elevated for the life of the flock.

A NATURAL OUTBREAK OF TOXOPLASMOSIS IN A BACKYARD FLOCK OF GUINEA FOWL IN MISSISSIPPI

[A]Kelli H. Jones, DVM, MAM, ACPV; [A]Floyd D. Wilson, DVM, BS; [B]Scott D. Fitzgerald, DVM, PhD, ACPV ; [B]Matti Kiupel, PhD, MS, BS, DACVP

[A]Poultry Research And Diagnostic Laboratory, College Of Veterinary Medicine, Mississippi State University
[B]152 Diagnostic Center for Population and Animal Health, Michigan State University

It is commonly known that *Toxoplasma gondii* affects most warm blooded animals, including many avian species. Past studies describing *Toxoplasma gondii* infections in the guinea fowl have only been experimentally induced. This is a case report of a naturally occurring infection with *Toxoplasma gondii* in a backyard flock of guinea fowl in north Mississippi. To our knowledge, this is the first report worldwide of a natural clinical infection in a flock of guinea fowl. Around the time of the investigation, the flock owner lost seven out of approximately twenty guinea fowl. Birds reportedly exhibited no clinical signs prior to death. Necropsy examinations were performed on two of the dead birds. There were no gross lesions, however, intra-lesional protozoan cysts suggestive of *Toxoplasma gondii* were observed microscopically. The organisms were confirmed via immunohistochemistry and PCR. In addition to positive birds, a barn kitten approximately four months of age tested positive for antibodies to *Toxoplasma gondii* via the indirect fluorescent antibody test (IFA). Given the life cycle of *Toxoplasma gondii,* in that felids serve as the only definitive hosts, feces from the positive kitten could have potentially served as the source of infection for the guineas.

11033

Increased Mortality and Lameness in Male Broiler Breeders Prior to Peak Production associated with Staphylococcus aureus

Michael P. Martin, Andy McRee, Kabel M. Robbins, Luke B. Borst, Kevin L. Anderson, Paula Jay, and H. John Barnes
North Carolina State University, College Of Veterinary Medicine, 4700 Hillsborough St., Raleigh, Nc., 27606

Multiple integrators in North Carolina have recently experienced increased male broiler breeder mortality and lameness prior to peak production. Although low levels of male mortality and lameness are common in broiler breeder flocks, male mortality and culling up to 3.5%/week due to lameness has been observed recently on several farms. Mortality typically peaks around 25 weeks of age. There does not appear to be any breeder strain predilection, however, different breeder strains can have different clinical presentations.

The results of field investigations into the increased mortality and lameness will be presented. This will include the clinical and epidemiological presentations, description of gross lesions, and results of phenotypic and genotypic analyses of the Staphylococcus aureus isolates.

Diagnosis: MG/MS in a Backyard Flock. Is Control Possible?

Patricia s. Wakenell and Daniel Wilson
Purdue University Animal Disease Diagnostic Laboratory

In Indiana there have been a number of diagnoses of MG and/or MS in backyard flocks of multiple species. We have determined the likely source flocks in 2 separate outbreaks, one involving commercial turkeys. With the exponential increase in backyard flocks and the mobility of these flocks, disease containment is difficult at best. We will be discussing the 2 above cases and what strategies did or did not work.

11035

vvNDV in South MS?

Philip A Stayer, Floyd D Wilson, Jackson L McReynolds
Sanderson Farms

Dead pullets from three and five week old breeder replacement flocks were taken to veterinary diagnostic laboratory. There were no gross lesions reported by the attending pullet supervisor. Diagnosticians reported full thickness mucosal erosions at the junction of the proventriculi and gizzards as well as streams of dark substance in proventriculi of dead birds, potentially reduced hemoglobin from mucosal bleeding. Differential diagnoses included viscerotropic velogenic NewCastle Disease, very virulent Infectious Bursal Disease and Avian Influenza. After implementing heightened biosecurity practices, further specimens were collected on site and sent to other supporting laboratories. Final diagnosis was not a notifiable disease.

A Good Case for Insomnia: Bedbug Infestation in Broiler Breeders

Sue A. Hubbard[1], Jason Cater[2], Danny L. Magee[1], Jerome Goddard[3], Kristine T. Edwards[3]

[1] Poultry Research & Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University[2] Cater Veterinary Services [3] Department of Entomology and Plant Pathology, Mississippi State University

Bedbugs have made a successful comeback recently in the United States. In the past several decades bedbug infestations being commonly found on a poultry farm was virtually unheard of in the United States. A commercial broiler breeder farm was discovered to be infested with this external parasite. The bedbug infestation was so severe that mortality and egg production were significantly affected. Although bedbugs historically have been known to prefer dark areas, they were visibly noticeable throughout this house during daylight hours. The diagnosis, treatments, management changes and follow-ups will all be discussed in this presentation.

11037

Necrotizing Hepatitis in Pigeons due to Toxoplasma gondii

Richard M. Fulton, Matti Kiupel
Michigan State University

A pigeon owner fed his flock on a Thursday afternoon and did not notice anything wrong. In the evening, three birds were found dead. The next day, six birds were found dead. Saturday, six more birds died. Sunday another bird died. A different flock of pigeons which was located only 25 feet away from the affected flock had no signs of illness or death loss. The owners were concerned that someone may have poisoned this flock. Dead birds, two each day, from Friday, Saturday and Sunday were brought to the diagnostic laboratory on Tuesday. Grossly, all most all (5 of 6) had hepatitis with red and yellow mottling. Half of the birds, in addition to mottling, had diffuse 1 mm random foci of necrosis. Histologically, the birds had a multifocal necrotizing hepatitis with intralesional protozoal organisms. The protozoan parasites were identified as Toxoplasma gondii by immunohistochemistry.

Avian Encephalomyelitis in Pen-Raised Bobwhite Quail

Douglas A. Anderson
Georgia Poultry Laboratory Network, Pobox 20, Oakwood, Ga 30566

This report details the clinical signs and diagnostic testing that resulted in a diagnosis of Avian encephalomyelitis in Bobwhite quail breeders and their progeny following a transient drop in egg production and accompanied by neurological signs and excessive mortality in hatchlings.

Infectious Coryza in a Commercial Broiler Breeder Flock

G. Donald Ritter
Mountaire Farms Inc., Po Box 1320, Millsboro, De 19966 Dritter@mountaire.com

Infectious Coryza is rarely diagnosed in commercial poultry flocks in the United States. This case report describes a case of infectious Coryza in a flock of 30 week old commercial broiler breeders. Disease background, discovery, symptoms, clinical progression, lab tests, treatment, epidemiology and economic outcomes to both the broiler company and farm family contract grower will be discussed in detail.

11040

Ulcerative Enteritis-like Disease associated with Clostridium sordellii in Quail

H. L. Shivaprasad, Monique Franca and Rocio Crespo
Cahfs-tulare, 18830 Road 112, Tulare, Ca 93274

Ulcerative enteritis is an acute bacterial infection of various species of birds including chickens, turkeys and game birds such as quail, pheasants, grouse and chukar partridges caused by Clostridium colinum. It is also called quail disease because it is especially severe in quail characterized by sudden onset, rapid spread within a flock and mortality sometimes as high as 100 %. Lesions are characterized by multiple ulcers throughout the intestinal tract- including ceca and foci of necrosis and inflammation in the liver.

However, there have been a few reports of ulcerative enteritis-like disease in quail associated with Clostridium perfringens but no reports associated with Clostridium sordellii. Here we present ulcerative enteritis-like disease associated with C. sordellii. Three separate submissions of quail ranging in age from 14 weeks to 30 weeks experienced anorexia, lethargy, diarrhea, loss of weight and increased mortality. Postmortem examination of ten quail revealed mucosal ulcers primarily in the small intestine in most birds and foci of necrosis in the liver of a few birds. Anaerobic cultures of the intestine in most birds and from a few livers yielded C. sordellii.

C. sordellii is a Gram positive anaerobic bacterium that has been associated with enteritis, hepatitis, myositis and sudden death in humans and animals. It has been sporadically reported in birds associated with enteritis, hepatitis, omphalitis and cellulitis.

"Silent" Histomoniasis on a Brooder Farm

Mark Blakley, DVM
Butterball

Birds from two consecutive brooder farm placements on an all-in, all-out turkey poult contract brooding facility showed signs of Histomoniasis at 12 weeks of age in the first brood and 8 weeks of age in the subsequent brood after being moved to geographically separated finishing farms with no prior history of Histomoniasis. Birds had varied mortality with some barns exhibiting mortality as high as 40% or more.

Otitis Externa in Chickens due to Staphylococcus hyicus?

Richard M. Fulton, Jeffrey G. Hunchar
Michigan State University

A small, closed flock of leghorn chickens kept in a totally biosecure environment developed otitis externa. Biosecurity included controlled ventilation and temperature, individual caging, irradiated food and shower before entry. Otitis externa was characterized by crusting of the external opening and filling of the external ear canal with a yellow waxy exudate. In some cases, the exudate occluded the canal. Staphylococcus hyicus was isolated.

Hemorrhagic Proventriculitis and Ventriculitis in Layer Pullet Chicks

C. G. Sentíes-Cué[1], J. Kelly[1], B. R. Charlton[1], and G. Cutler[2]

[1]California Animal Health and Food Safety Laboratory System, Turlock-Branch
1550 N. Soderquist/ P.O. Box 1522. Turlock, CA, 95381

[2]Cutler International Associates

Increased mortality and weakness occurred in a 2-day-old, 100,000 layer pullet flock. Percent of mortality in the 1st and 2nd weeks were 2.6 and 1.3 respectively. At necropsy, multifocal hemorrhages were seen on the proventricular mucosa. The koilin layer of the ventriculus was eroded, ulcerative, and darkly stained in most of the birds examined. Microscopic examination of the koilin layer exhibited multiple clear spaces and uneven staining, as well as hemorrhages in the epithelium and muscular layers. The cause of the lesions was not determined. Possible disinfectants or water line flushing products accumulating in the cups of the nipple drinker system was the cause of toxicity. Another 100,000 layer pullet flock received in another house without cups in the nipple drinking system did not have the problem.

11044

Detailed Analysis of 2009-2011 ELISA Serological Data from the GA Poultry Industry

Louise Dufour-Zavala, Len Chappell, Brenda Glidewell
Ga Poultry Lab Network

The poultry industry routinely uses ELISA serology to monitor their flocks for the presence of undesirable agents (Mycoplasma, influenza), and to monitor flock exposure or vaccination status (IBV, NDV, REO, IBD, CAV, AE). Access to large amounts of data in breeders and broilers is enabling us to analyze the data considering several paparmeters such as age and vaccination program, and to establish baselines, critical in future interpretation of ELISA data for the poultry industry. The baselines and data analysis will be presented.

Genetics and the future of poultry production

J.C. McKay
EW Group, Newbridge, Midlothian, United Kingdom

Poultry have been domesticated for thousands of years and man has made many genetic changes during the process of domestication. The genetic progress made in the last eighty years has been the foundation of the modern poultry industry that is a major source of animal protein in most countries of the world. Improvements in health, nutrition and environmental management have all contributed to improved performance but genetic improvement has been a major driver for the development of poultry production.

Egg production has been improved consistently over seventy years and the industry continues to improve the efficiency of production by at least 1 per cent per year. In broilers, combined selection for growth, body composition, feed efficiency and livability continues to deliver 2-3% improvement per year in the efficiency of meat production.
Sustainable genetic programmes must manage genetic resources for long term improvements and give high priority to the health and welfare of the stock.
Improving efficiency of production reduces the overall environmental impact of poultry production and improves the sustainability of the industry. Disease challenges can have a major impact on efficiency but eradication of disease agents, and improved vaccination, nutrition and biosecurity have contributed to improved livability and welfare.

Continuing genetic improvement in disease resistance will be an important component of future genetic programmes. This must be true for both infectious and metabolic diseases. Closer collaboration between breeding companies and vaccine companies may produce more effective protection against the major diseases. Breeding programmes must have a sound ethical basis supporting continuous improvements in animal health and welfare and the management of genetic resources. They must also support more sustainable use of all the resources required to produce animal protein.

Future Considerations in Poultry Nutrition

Steve Leeson
Department of Animal & Poultry Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

The poultry industries have undergone remarkable change and growth over the last 30 yrs, and it seems that this trend will continue in the next 5-10 years. The meat industry has undoubtedly been the most successful, yet the egg industry is now making strides in new product development. Today, we see 3 kg male broilers at 40 d of age, while white egg layers are capable of producing in excess of 330 eggs in 52 weeks of lay. There is often debate about there being an end point to this increased genetic potential, yet the geneticists tell us that selection pressure will be little reduced in the foreseeable future. Feed will always be the major input cost for poultry meat and egg production. Corn and soybean meal will remain as the basis of all poultry diets, and for this reason the Americas` will continue its dominance in production. There are really no major alternatives to corn and soybean meal on a worldwide basis. In certain regions, wheat is used because of artificial price support and/or artificial import tariffs on alternative ingredients, and rice bran will continue to provide a niche role for diets in Asia. One often hears about 'new' alternate feed sources and by-products. Such discussion shows lack of understanding of world animal and crop production, and whereas a few hundred tonnes of a 'new' by-product can be fitted into some local production system, there simply is not any undiscovered ingredient that is going to have an impact on the price of corn and soybean meal. In this regard, DDGS is not a ‘new’ ingredient. Some type of HACCP program will become mandatory at feed mills and this will extend from concern over residue of pharmaceutical products through to microbial control. Undoubtedly the microbial status of poultry products in general is going to be the single largest factor influencing the success of future production systems. Feed is but one source of such potential contamination, and contrary to popular belief, elimination of meat meal from the formulation is no guarantee of producing feed devoid of pathogens to birds or humans. Few important microbes can withstand feed treatment at 80ºC, and so the current trend to higher pelleting temperatures. However recontamination following pelleting is still a major problem, and the use of organic acids/formaldehyde treatments etc. may become more popular to maintain 'clean' feed delivered to the feed trough. We will have to resolve the problem of sustaining eggshell quality in layers and breeders and bone integrity in ever-younger meat birds. We need a better understanding of calcium flux in the hen and calcification of the skeleton of young meat birds. In developed countries there will be more emphasis on meeting consumer demands, such as designer eggs and value-added meat products. Production will likely be less reliant on feed-borne antibiotics, growth promoters and anticoccidials, although not entirely so. All nutritional and production challenges will have to be considered in a situation where concerns for the environment are increasing, and where supplies of “clean” water become more critical.

A CRYSTAL BALL LOOK INTO THE FUTURE OF HATCHERIES AND HATCHING EGGS IN THE POULTRY INDUSTRY OVER THE NEXT 5 TO 10 YEARS.

Michael J. Wineland
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Genetic and nutritional advances in the poultry industry have resulted in a hatchling that has greater potential for improved growth and feed conversion than ever before. It is expected that this improvement will continue and will result in the incubation duration becoming a greater percentage of the total life span of meat type poultry species. Incubation concepts have not changed although recent research has found that management basics must change in order to attain the potential that advanced genetics and nutrition have provided us. Hatchery design is being altered to allow for changes made to egg and chick handling, improved incubation equipment and for better hygiene in the hatchery. Research is continuing by groups around the world into how incubation parameters alter the physiological maturation of different organ systems such as the gastro-intestinal tract, muscle, bone, endocrine, exocrine and the immune systems which will help us to understand how we must regulate incubation to achieve the hatchlings potential. In some cases we also do not see embryos that behave exactly the same when obtained from different primary breeders either because of specific genetic make up the embryo or because of differences in the eggshell that the embryo is developing within. This will require the development of specific profiles of incubation parameters for the different breeds. The poultry industry is realizing that hatcheries can be a profit center when incubation is managed to optimize hatchling potential. Research is being conducted that will allow operations to manipulate incubation so as to prepare the hatchling to perform better under specific environmental conditions during grow-out. To accomplish the improved management of the developing embryo the hatcheries will use variations in single stage incubation concepts. There will be procedures developed that will allow incubators to sense changes in the embryo and allow for alteration of growth and development and influence hatch window. There will be continued development of electronic controls and robotics to provide for labor savings in the hatcheries. Procedures are being developed that will determine how best to handle eggs from the time they are laid through the period prior to incubation so as to minimize hatch loss from stored eggs. This will be accomplished by using techniques that provide for a more viable embryo prior to egg storage or by better maintaining the various components of an egg when it is stored for varying lengths of time. As the understanding of the developing embryo becomes clearer between the time the egg is laid until the hatchling is removed from the hatcher, changes to egg handling and incubation management will continue.

11049

Vaccination and immune modulation in poultry to mitigate infectious diseases and antibiotic use

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As the world population grows and developing countries become more affluent, the global consumption of meat will increase by more than 50% within the next 10 years. Confronting the increased demand for poultry food products are emerging field diseases, increasing regulatory bans of antimicrobial growth promoters, high-density growth conditions, and waste management. Although biotechnology offers solutions to some of these challenges, basic studies are needed to better understand the complex interaction between the intestinal microbiome, host immunity and the environment. This presentation will focus on emerging strategies to enhance gut immunity and to decrease economic losses due to poultry diseases. The interaction of the intestinal microbiota with the host, improved vaccines to stimulate broad-spectrum immunity, development of novel adjuvants and diagnostic tests for host immune status, and enhancement of immunity using dietary supplementation with genetically engineered antibodies, probiotics, antimicrobial peptides and immunoactive phytochemicals will be discussed.

11050

Coccidiosis Control: Today and In the Future

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Coccidia are protozoan (Eimeria spp) parasites (9 chicken species) that infect the intestine or ceca of poultry which negatively impacts production with losses in feed conversion, weight gain, uniformity, pigmentation, and increased mortality. Polymerase Chain Reaction (PCR) technology has shown that E. acervulina and E. maxima are the most common species followed by E. tenella. Advances in PCR technology will allow for the ability to track not only species but strains of Eimeria. Poultry coccidiosis is currently controlled by vaccination or the use of prophylactic feeding of drugs. Both types of anticoccidial programs rely on immunity development. Lack of total control due to drug resistance and/or the mode of action allow coccidia to cycle. This stimulates immunity development. Vaccination programs use live oocysts that are administered using a spray or gel in the hatchery, a gel puck placed into the chick box, or in-ovo dosing. These methods provide a prescribed amount of oocysts at an early age enabling immunity development to progress rapidly while at a desired rate. Two types of coccidia are used in vaccines: non-attenuated or attenuated (low pathogenicity). Prospects for improving application methods, examining new methods of attenuation (such as radiation), combining of non and attenuated strains, and developing sub-unit vaccines will be a critical part of future coccidiosis control. Other keys to future coccidiosis control will be to gain a better understanding of the epidemiology of the coccidia, interactions with other diseases, relationship of nutrition and coccidiosis, and genetic selection for stronger immunological competence or resistance.

11054

Transforming Bird Health and Managerial Considerations into Caloric Costs in the Production Environment

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Non nutritional factors that affect ADG and FCR (such as pellet quality and ambient temperature) can be converted into calorie equivalents known as "Effective Caloric Value" (ECV). At a given feed cost, these calorie equivalents can be translated into tangible economic values. The ECV system has been expanded to also include aspects of bird health (immunity development) and disease consequence (maintenance and malabsorption) that strongly influence profitability. Calories are expressed as dietary caloric density and enable contrasts of the commercial production environment with bird genetic potential under more ideal conditions. The ECV system also provides a target for bird energetic efficiency and a method by which added costs may be identified. As example: ECV application for production of 2.5 kg birds establishes the genetic potential as requiring the input of 13,676 kcal ME. Further, evaluation of a commercial broiler production system indicated the routine input of 15,227 kcal to achieve the same 2.5 kg mass. ECV analysis of the production environment suggested that the 1,551 additional kcal ME input was attributable to 427 kcal reduced pellet quality, 214 kcal lighting program cost, 290 kcal as ambient temperature related and assuming a lesion score (upper=1; mid=2; cecal=0.7) 6 days prior to processing as 498 kcal totaling 1,429 kcal ME. In this case the ECV approach suggested an accounting for 92% of the calories wasted over bird genetic potential. In summary, the ECV system provides the opportunity to express a broad array of factors impacting bird performance as calorific and economic cost.

11055

Pathology Associated with a Chicken Astrovirus Isolated from Broiler Chickens with Runting and Stunting Syndrome

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Chicken Astrovirus CAstV-4175 was isolated, purified, titrated and inoculated into chicken embryos and day-old chickens alone or in combination with orthoreovirus REO-4175. Both viruses were originally isolated from young commercial broilers affected with Runting and Stunting Syndrome (RSS). CAstV-4175 infection induced villus shortening and clubbing in the duodenum and jejunum, and severe enterocyte vacuolar degeneration. CAstV-4175 infection was localized in the distal villus enterocytes and induced significant growth retardation. REO-4175 alone induced growth retardation as well and feather abnormalities. Virus localization, and microscopic and ultrastructural pathological changes were documented using in situ hybridization, light microscopy, transmission electron microscopy and scanning electron microscopy.

Comparative Pathology of Diseases of Tendons in Broilers and Broiler Breeders

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Tenosynovitis and end-stage tendon failure in the legs of broilers and broiler breeders is a cause of lameness and culling. The most common diseases that affect tendons in heavy breed chickens are bacterial tenosynovitis, viral tenosynovitis (viral arthritis) caused by reovirus, and tendonopathy (tendonosis), a degenerative condition. Representative gross and microscopic lesions will be presented for each condition, as well as perspectives on pathogenesis and relative incidence in broiler production.

Molecular characterization of *Mycoplasma gallisepticum* isolated from Chicken and Turkey

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Mycoplasma gallisepticum (*MG*) infection in chicken and turkey is still one of the important reasons causing economic losses in poultry. The current study concerned with rapid detection and molecular characterization of *MG* isolates. The all samples positive by culture were positive by PCR and rt-PCR. Five isolates (four from chicken and one from turkey) were sequenced for *mgc2* gene. The present molecular study demonstrated four wild-type *MG* strain. (Eis 3- C-10, Eis 4- C-10, Eis 5- C-10 from chicken Eis 6- T-10 from turkey) isolated from diseased chicken and turkey flocks. While Eis 7- C-10 (vaccinal F- strain) was isolated from commercial layer flock vaccinated with F- strain vaccine. We concluded that *mgc2* gene was able to distinguish between *MG* wild - type and vaccinal strains.

11057

The Efficacy Two Mycoplasma gallisepticum Vaccines in Laying Hens

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A significant percentage of Mycoplasma gallisepticum (MG) vaccine doses administered are used to prevent the clinical effects of MG in long-lived birds and their progeny. However, vaccine efficacy studies that evaluate egg production losses and vertical transmission of MG are lengthy and expensive. In this study we used the incidence of ovarian regression (follicle atresia) as an indicator of protection of the reproductive tract by ts-11 and a new vaccine K-strain. Vaccinated layer-type chickens in egg production were challenged with virulent R-strain at 25 weeks of age and evaluated at 10 days post challenge. Both ts-11 and K-strain vaccination resulted in a significantly decreased incidence of ovarian regression, as well as significantly reduced air sac and tracheal lesions. The vaccines also reduced the colonization of the trachea with R-strain.

Tracking Infectious Laryngotracheitis CEO Vaccine: Field to Processing

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Summary: Infectious Laryngotracheitis (ILT) persists as a severe respiratory problem for poultry worldwide. ILT chicken embryo origin (CEO) vaccine is considered a source of vaccinal laryngotracheitis . It has been suggested that stress associated with broiler catching and transportation causes birds to shed CEO vaccine virus; hence, spreading to other poultry in route to processing plants. The goal of this research was to document possible shedding of CEO vaccine virus from 14 days post vaccination to processing. Tracheal swabs were obtained at intervals post vaccination and qPCR was used to quantify the CEO viral load in the trachea of vaccinated chickens.

COMPATIBILITY OF VECTORED LT VACCINES AND VECTORMUNE HVT NDV

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Biomune Company does not promote the mixing of vaccines. However, it is known that this occurs under normal field conditions. Multiple studies were conducted to evaluate the compatibility of administering HVT or various vectored vaccines (Vectormune HVT LT, Vectormune HVT NDV, or Vectormune FP LT) at the same time. Compatibility of these vectored vaccines was based on protection after challenge with ILTV (infectious laryngotracheitis virus) or NDV (Newcastle disease virus), B1B1 strain. For *in ovo* administration the vaccines were applied to chicken embryos at 18 days of incubation or for subcutaneous route the vaccines were applied at day of age. The results demonstrated vaccine interference when administering vectored vaccines utilizing the same vector but not when administering vectored vaccines using different vectors.

11060

The Effect of Vectored HVT+IBD (Vaxxitek Â® HVT + IBD) Vaccination on Body Weights, Uniformity and Virus Shedding in Commercial Broilers

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The introduction of vectored HVT+IBD vaccines has offered an alternative to traditional programs. The current study compared the effect of a vectored HVT+IBD vaccination to MAb on body weight, flock uniformity, and virus shedding in a commercial broiler flock.

A flock of day-old broiler chicks were divided into 2 equal groups and placed in a commercial broiler house. The control group was administered Marek s disease vaccines in ovo at 19 days of incubation. The control group was not vaccinated for IBD. The treated group was administered vectored HVT+IBD in ovo at 19 days incubation.

The flock was weighed at five time points. At least 200 birds were individually weighed in each test group at each time point. Mean weights and CVs were calculated for each test group. Flock uniformity was calculated as the number of birds within a range of +/- 15% of the mean weight.

At each time point cloacal swabs were collected from 90 birds per group and tested for the presence of IBD virus using rt-PCR. Serum samples were collected from 25 birds per group and tested for IBD antibodies using ELISA. Bursal tissue samples were collected from 6 birds per group. Fresh bursal tissue was tested for IBD virus using AC-ELISA. Formalin-fixed bursal tissues were examined for histopathological lesions of IBD.

At 38 days of age, 120 birds per test group were selected for processing. Mean weights and CVs for each test group were determined for live weight, RTC weight, and cut up parts weights (wings, breasts, tenders, legs and racks). Yields were calculated as a percentage of live weight and RTC weight.

The treated group had a higher mean weight, lower CV and better uniformity than the control group. The treated group had lower mean bursal scores and better follicular restitution. The treated group had higher geometric mean titers on ELISA at each time point. More birds in the control group were shedding virus at 28 and 35 days. The treated group had higher live, RTC, and cut-up parts weights and higher yields for RTC, wings, breasts and tenders.

11062

Use of CVI988 in Optimizing Revaccination Protocols Against Marek's Disease

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Marek's disease (MD) continues to be one of the major threats for the poultry industry. We have recently demonstrated that in ovo vaccination with HVT followed by administration of HVT+SB-1 at hatch improved protection. However, such protection is not enough against early challenge with very virulent plus Marek s disease virus (MDV). In the present study we have evaluated the positive effect of revaccination when CVI988 is used. Our results show that in ovo administration of either HVT or HVT+SB-1 followed by administration of CVI988 at day of age protects better than single administration of CVI988 in ovo or at day of age, and constitutes therefore the most protective vaccination protocol currently available.

11063

Effects of Interspecies Adaptation to Different Poultry on a Wild Bird Origin H5N1 Low Path Avian Influenza Viral Genome

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A domestic low pathogenicity (LP) H5N1 avian influenza virus (AIV), A/mallard/Maryland/1159/2006, was serially passaged 10-times in turkeys, quail, and chickens to adapt the virus to each species. As a control, the virus was also passaged 10-times in mallard ducks. A whole genome cDNA sequencing library was prepared from the original duck origin H5N1 viral RNA for use with the Illumina Genome Analyzer IIx, an ultra high-throughput sequencing system based platform. One-hundred-thirty cycle, paired-end sequencing reads were compiled in a de novo alignment using the DNAStar NGen sequence assembly software. Single nucleotide polymorphism (SNP) analysis identified minimal gene variation in the original viral stock. Additional cDNA libraries were created for high-throughput sequencing using the 10th passage of each host adapted LPAI. Forty-two cycle, single-end sequence reads from the species adapted libraries were compiled using the original duck origin H5N1 viral gene sequences as a template. The observed genetic variability discovered in the species adapted isolates compared to the original duck origin virus will be presented.

11064

Alteration of a Single Amino Acid in the Basic Domain of Marek's Disease Virus Meq Oncoprotein Plays an Important Role in T-cell Transformation

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Marek s disease virus encoded oncoprotein, Meq, has been shown to play a major role in transformation of T-lymphocytes. We have earlier shown that replacement of the meq gene in the very virulent strain Md5 with that of vaccine strain CVI988/Rispens resulted in virus attenuation in chickens. To determine the role of individual amino acid residues in the transformation of T-lymphocytes in chicken, we generated a mutant rMd5 virus in which amino acid residue at position 71 was changed from alanine to serine (A71S). This recombinant virus replicated in vitro to levels similar to parental rMd5 virus; however, it was shown to be poorly oncogenic in chickens suggesting amino acid alanine at position 71 in the DNA binding domain of the Meq protein plays an important role in Marek s disease oncogenesis.

11065

Evaluation of Cytokine Gene Expression After Avian Influenza Virus Infection in Avian Cell Lines and Primary Cell Cultures

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The innate immune responses elicited by avian influenza virus (AIV) infection has been studied by measuring cytokine gene expression by relative real time PCR (rRT-PCR) in vitro, using both cell lines and primary cell cultures. Continuous cell lines offer advantages over the use of primary cell cultures, but it s not clear if the results are comparable when studying the innate immune responses to virus infection. In this study, the cytokine gene expression and viral replication in two chicken-origin cell lines, DF-1 (fibroblast) and HD-11 (macrophage), was compared to two primary cell cultures, chicken embryo fibroblast (CEF) and peripheral blood mononuclear cells (PBMC) at 2, 8, and 24 hours post infection with a low pathogenic avian influenza virus, TK/WI/68 (H5N9).

11066

Correlation between Marek's Disease Virus Replication Rates and Pathotype Based on Fifteen Virus Strains

John R. Dunn
Usda-ars-adol

Previous studies have suggested a relationship between replication rates and virulence of Marek's disease virus strains. In most cases, past studies have compared a single vv+MDV strain to a vMDV strain. The purpose of this study was to confirm a correlation between replication rate and pathotype using a broad selection of virus strains. In vivo replication was evaluated for 15 strains, including 5 vMDV (JM/102W, GA/22, 571, 596A, and 617A), 5 vvMDV (Md5, 549A, 568A, 595, and 643P), and 5 vv+MDV strains (584A, 645, 648A, 660A, and 686). Results from qPCR and virus isolation will be discussed.

MAREK'S DISEASE VIRUS INDUCED TRANSIENT PARALYSIS – A CLOSER LOOK

Mohammad Heidari and Ming Xu

Marek's Disease (MD) is a lymphoproliferative disease of domestic chickens caused by a highly cell-associated alpha herpesvirus, Marek's disease virus (MDV). Clinical signs of MD include depression, crippling, weight loss, and transient paralysis (TP). TP is a disease of the central nervous system, which affects the MD-susceptible chickens 8-11 days post inoculation (dpi), normally resulting in death 1-3 days after the onset of clinical sign. Chickens from lines 7_2 (MD-susceptible) and 6_3 (MD-resistant) were inoculated with a highly pathogenic strain of MDV at one week of age and brain samples from birds with and without TP were collected at 5, 11, and 21 dpi for cytokine and other immune-related gene expression analysis and computation of viral genome copy number. Data revealed that chickens inoculated with MDV had higher levels of IL-6, IL-10, IL-18, IFN-α, IFN-β, IFN-γ, MHC I, and CD-18 in their brain tissues at 11 dpi compared to the uninfected control birds. In addition, the expression levels of IL-6, IL-10, IFN-α, IFN-β, and IFN-γ were significantly higher in the brain tissues of the birds showing clinical signs of TP in comparison to MDV-infected chickens with no TP. The genome copy number of MDV was also significantly higher in the brain tissues of the infected birds with clinical TP than those without TP. Comparative analysis between the two chicken lines showed that the expression levels of IL-6, IL-10, IFN-β, IFN-γ, IFN-λ, IL-18, CD-18, and MHC I were significantly higher in the brain tissues of the birds from line 6_3 with TP than those of line 7_2 exhibiting clinical signs of TP. The expression pattern of these immune related genes suggests a possible immunological mechanism for the differential responses of the two chicken lines to MDV infection and exhibition of MDV-induced TP.

11069

Appraisal of Experimental and Commercial Marek's Disease Vaccines to Induce Bursal and Thymic Atrophy

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Recently, several experimental Marek's disease (MD) vaccines were developed that appear to protect equally or better than the best commercial vaccines. However, some of the experimental vaccines were reported to induce transient bursal and thymic atrophies. We will report on two promising experimental MD vaccines, documenting the incidence of bursal and thymic atrophy, as well as their ability to inhibit weight gain. To determine whether there is a correlation between virus replication and lymphoid organ atrophy, we also quantified the Marek s disease virus present in the thymus at various times post-inoculation.

11070

Chronological Study of the Pathogenesis of Oncogenic and Attenuated Marek's Disease Virus Strains in the Lung

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The pathogenesis of serotype 1 Marek's disease virus (MDV) strain 648A at different passage levels was evaluated in the lung. One-day-old SPAFAS chickens were inoculated subcutaneously with 2,000PFU of either the very virulent plus 648A-10 or the attenuated strain 648A-80. Lungs were collected at 3, 5, and 10 days after infection (dpi) for nucleic acid extraction and for immunohistochemistry. Pathogenesis of MDV was evaluated by various criteria (1) Replication of MDV; (2) infiltration of inflammatory cells; (3) expression of major histocompatibility complex class II; and (4) various cytokine transcripts. The role of lung in the pathogenesis of both vaccine and oncogenic MDV will be discussed.

11071

Measurement of CD4, CD8, Class II, and Macrophage Antigen Expression in Chicken Lungs

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Determination of antigen expression in chicken lungs is used to assess responses to viral infection including studies on the pathogenesis of Marek's disease herpes virus, and responses to Marek's disease vaccine viruses. Comparison of manual counting of positively stained CD4 and CD8 cells correlated well (r = 0.779 for CD4) with total area of antigen expressed as measured by imageJ using thresholded digital images. The procedures were applied to measure total area for Class II and macrophage antigen expression and are more reliable and reproducible than estimating antigen expression when positive cells are too numerous to count.

11072

Replication of Swine-lineage Influenza Virus in Juvenile and Adult Turkey Hens

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Since the first reported isolation of swine influenza viruses in turkeys in the 1980s, transmission of swine influenza viruses to turkeys has continuously been documented. In the majority of cases, the turkey flocks were housed in close proximity to swine herds. In addition, the 2009 pandemic H1N1 virus, which is thought to be a swine-origin, was detected in turkeys with a severe drop in egg production. However, experimental infection of turkeys with pandemic H1N1 influenza viruses was not successful in more than one trial. In this study, in addition to young turkeys (2-4 weeks old), we experimentally infected turkeys hens (48-week-old) with A/swine/Ohio/24366/07 (H1N1) strain that was transmitted to humans during a county fair in the state of Ohio. Oviduct samples in addition to tracheal and cloacal swabs were collected and tested with real time RT-PCR and virus isolation. Sera were collected at 14 days post inoculation and tested for the presence of influenza antibodies. In juvenile turkeys, we were not able to detect the virus from tracheal and cloacal swabs although the birds seroconverted. In layer turkeys, small amount of viruses were detected from tracheal and cloacal swabs (<2.2 EID50/ml), and greater amount (>3.5 EID50/ml) of viruses in all different parts of the oviduct. We speculate that turkey hens at the early stage of egg production (28-32 weeks of age) could be affected more severely from swine-lineage influenza virus infection.

11073

Global Gene Expression Analysis to Compare Intestinal Transcriptional Responses against Three Major Eimeria Species, E. acervulina, E. maxima and E. tenella

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This study was conducted to compare the differential transcription response of broiler chickens following oral infection with three major Eimeria species, E. tenella, E. acervulina or E. maxima using avian intestinal intraepithelial lymphocyte cDNA microarray (AVIELA). The transcriptional modification after primary and secondary infections of young broiler chickens with each of three Eimeria species was examined using freshly obtained intestinal intraepithelial lymphocytes (IEL) from coccidian-infected broiler chickens to gain better understanding of host-pathogen immunobiology. Generally, there were less changes in gene expression following secondary infection compared to primary infection for each Eimeria species and limited numbers of differentially expressed genes were found between early and late stages of secondary infection. Among the three Eimeria species that were tested, E. tenella infection induced the least alteration in transcription between primary and secondary infections even though E. tenella provoked the most changes in the number of altered genes compared with non-infected control. Gene Ontology analysis revealed that the genes associated with metabolism pathways being the major genes changed (>2.0 fold) following the infection with three Eimeria species. Pathway analysis identified several biological functions altered by different Eimeria infection. The information obtained from this study will expand our understanding of host-pathogen immunobiology and will enhance our ability to develop new control strategies against avian coccidiosis.

Natural Infection of H5N1 Avian Influenza in Budgerigars and Zebra finches

Ghada S. Moussa
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Cases of H5N1 avian influenza were diagnosed in some pet birds, including Budgerigars, Cocktails, Zebra finches and Rouen ducks. Budgerigars, cocktails and finches showed signs of conjunctivitis and mild respiratory distress, while some rouen ducks showed nervous and paralytic symptoms. Histopathology revealed severe acute exudative interstitial pneumonia, heterophilic and mononuclear infiltrates, odema, hemorrhage and thrombosis. Liver showed focal areas of necrosis, karyorrhexis and karyolysis of the nuclei of hepatocytes as well as congestion and dilatation of sinusoids with thrombosis. Heart showed severe congestion and hemorrhages. Infection was proved by common A and H5 Rapid antigen capture immune-assay (AC-EIA).RT- PCR. In conventional PCR using specific four primers for full sequences of H5 gene, the amplification products were identified at the expected molecular weights of 533 bp, 505 bp, 533 bp and 420 bp.

A New Species of Eimeria (Apicomplexa: Eimeriidae) of Turkeys (*Meleagris gallopavo)*

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A new species of coccidia is described for the turkeys. Feces were from commercial turkey operations throughout the US and wild turkeys from the Delmarva. Eimeria edgari sp.n. is proposed. Sporulated oocysts are subspherical, measuring 17.824 x 16.445 µ (16.915-19.549 x 14.585-18.314 µ) with a shape index of 1.0839. The parasites invade the epithelial cells from the duodenum to the ileum; parasites were on some occasions found in the epithelial cells of the ceca and rectum. Parasitism is accompanied by severe mucus production in the upper half of the small intestines. Gross lesions are hyperemia, excess mucus in the duodenum and jejunum also watery, mucoid and bloody feces. Poults hyper-immunized with *E. meleagrimitis, E. adenoeides* and *E. dispersa* and challenged with the suspect organisms (*E. edgari*) exhibited clinical signs of depression and lethargy by 6.5 days post challenged. Poults developed immunity following several low grade exposures and immunity was only to *E. edgari.* This species invades the same regions of the intestines as *E. meleagrimitis* and *E. dispersa*, but the oocysts of *E. edgari* are smaller than those of these two species. Birds immunized with known Eimeria species not protected against *E. edgari* and visa versa. This species appeared to be relatively prevalent, found in many of the turkey growing areas in the US.

Nicarbazin: Field Experiences Improving Intestinal Health

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The carbanilide anticoccidial Nicarbazin is one of the most effective tools available at this moment to safely prevent and control coccidiosis in broilers during high challenge situations. Many common anticoccidial combinations and coccidiosis control strategies benefit from the excellent performance of Nicarbazin in the field. The most recent field experiences and retrospective studies will be discussed in detail to adequately inform attendees how to get the most benefits from Nicarbazin-based programs.

Case Study: Economic Consequences of Weather and Coccidiosis in One U.S. Broiler Integration

Linnea J. Newman, Robert Teeter
Intervet/schering-plough Animal Health

Broiler integrators evaluate production costs on a weekly basis. But with each week, many variables affect production cost such as feed cost and conversion, weather and disease challenge. This case study utilizes a 3-year weekly performance history with regional weather data and coccidiosis control program data to isolate the variables of weather and coccidiosis. We estimate an economic consequence for these variables under real-world conditions. The economic estimates are compared to effective caloric value data derived from controlled calorimetry chamber studies at Oklahoma State University. Understanding economic consequences of specific variables enables integrators to evaluate investments to control these variables.

Working with Coccivac D in a Dry Climate

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Coccidial vaccination has been widely employed with good success in many broiler breeder operations. In Alberta, Canada previous attempts to use vaccination successfully have failed. A traditional approach to encourage successful coccidial cycling and therefore successful vaccination is to increase bird density. Density can only be adjusted to a certain extent however, before there is a negative impact on broiler breeder pullet uniformity. Investigation into underlying causes for the previous unsuccessful vaccinations has revealed that low humidity may have played a role. Recent field experience using coccidial vaccination in an environment to which moisture has been added will be presented.

11079

An Alternative Method of Delivering Eimeria Oocyst Vaccines to Protect Against Avian Coccidiosis

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Avian coccidiosis in broilers is controlled by either anticoccidial drugs in feed or administration of live Eimeria oocyst vaccines. Our research indicates that traditional methods of vaccine administration may be inefficient such that a significant number of chicks are not immunized at an early age, and thus remain susceptible to coccidiosis during grow-out. An alternative delivery method, namely administration of a mixture of Eimeria oocysts suspended in semi-solid gelatin pellets and applied directly to feed is being tested by measuring oocysts output and protective immunity against Eimeria challenge. Preliminary studies revealed that Eimeria oocysts are stable in gel pellets, and chicks excrete oocysts within 4 days after ingesting gelatin/oocysts- containing feed. This approach may represent a way to ensure more uniform vaccination against coccidiosis.

11080

Attenuation of Development of Eimeria maxima following Gamma Irradiation

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Previous research demonstrates that infecting chicks with irradiated oocysts attenuates normal disease development but results in immunity to challenge infection suggesting that irradiated oocysts are a viable substitute for conventional live vaccines to control avian coccidiosis. Due to increased interest in use of live vaccines, the current study examines the effect of irradiation on the basic biology of a pathogenic isolate of Eimeria maxima. The results indicate a curve linear relationship between radiation dose levels of oocyst exposure, pathology, and oocyst excretion providing additional evidence that irradiation of oocysts may be useful as an alternative to conventional vaccines.

11081

Effect of Montanide ISA 71 VG on Recombinant Coccidia Antigen Vaccination

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A study was conducted to investigate the effects of Montanide adjuvants in emulsion with a recombinant protein in a vaccination trial against avian coccidiosis. Chickens were immunized by subcutaneous injection with a purified Eimeria recombinant protein, either alone or mixed with Montanide™ ISA 70 VG or ISA 71 VG. Body weight, fecal oocyst shedding, intestinal cytokine responses were evaluated before and after oral challenge. Intraepithelial lymphocytes activated by vaccination with Montanide™ ISA 71 VG formulation were isolated at D23 and tested for their ability to be stimulated by CON A or profilin. Cross protections conferred by Montanide™ ISA 71 VG based formulation was also assessed using E.acervulina and E.tenella strains. Immunization with Montanide™ ISA 70 VG or ISA 71 VG increased ($P < 0.05$) body weight. Recombinant protein formulated with Montanide™ ISA 71 VG also reduced oocyst shedding. Increased levels of mRNAs coding IL-2, IFN-γ were observed while levels of IL-10, IL-15 were reduced in the intraepithelial lymphocytes. Increased infiltration of CD8+ and TCR+ lymphocytes in injection site was observed as well as an improved capacities of intraepithelial lymphocytes to be in vitro stimulated by CON A and profilin. After challenge, reduction of parasite excretion in feces as well as preserved body weight gain whatever the Eimeria strain used demonstrated a cross protection induced by the tested vaccines. These results suggest that vaccination with the E. acervulina recombinant protein in combination with Montanide™ adjuvants triggers protective cellular immunity against avian coccidiosis.

The Synergistic Effects of Plant-Derived Nutritional Mixtures on Recombinant Antigen Vaccination against Avian Coccidiosis

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The present study was conducted to examine immunomodulatory effects of commercially available dietary plant-derived phytonutrients mixture (XT) on adaptive host immune response against avian coccidiosis. XT is a nutritional mixture of 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin. Vaccination against avian coccidiosis was carried out by subcutaneous immunization of young broiler chickens using recombinant Eimeria profilin protein at day 7 after hatch and challenge infection was given by an oral inoculation using live sporulated oocysts of E. tenella (ET) (2.0 Ã— 104) at 17 days of age. Four groups of broiler chickens (12 birds/group) were continuously fed a standard diet without (CON) or with profilin vaccine (CON-V) or standard diet supplemented with XT with profilin vaccine (XT-V) for 23 days. Changes in body weights were measured at 9 days post-infection (DPI) and fecal oocyst outputs were assessed in individual samples collected from 5 through 9 DPI. Cell-mediated immunity was assessed by evaluating the cecal cytokine transcript levels of IFN-Â³, IL-6, IL-17, and TL1A by quantitative real time-PCR at 0 DPI. XT-V group showed 20% increase in body weight ($P<0.05$) in comparison to the CON group after ET challenge infection. Fecal oocyst shedding was significantly reduced by 35% in XT-V group compared with the infected CON group. Furthermore, IFN-Â³, IL-6, IL-17, and TL1A cytokines were significantly decreased in XT-V group in comparison to the CON group. This study demonstrates that molecular and cellular changes were affected by XT-nutritional immunomodulation with enhanced vaccine-induced protective immunity against avian coccidiosis.

Immunopathogenesis of Infectious Bursal Disease Virus in Chickens

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This study was conducted with an aim to evaluate the innate and adaptive immune responses of infectious bursal disease virus (IBDV). We inoculated 3-week-old SPF chickens with either classic IBDV (cIBDV) or variant IBDV (vIBDV). At post inoculation days (PIDs) 3, 5 and 7, gene expression was determined in the bursa of fabricius (BF) by quantitative RT-PCR. There was upregulation of innate cytokines (IFN-Â± and IFN-Â²), proinflamatory cytokines (IL-6 and IL-1-Â²), chemokines (IL-8 and MIPÂ±) and iNOS in cIBDV- and vIBDV-infected BF. The expression of toll like receptor-3 (TLR-3) was downregulated at PIDs 3, 5, and 7 in the BF of vIBDV-infected chickens whereas TLR-3 was upregulated at PIDs 3 and 5 and was downregulated at PID 7 in cIBDV-infected chickens. We also evaluated the molecular mechanisms of cytotoxic T cell responses in the pathogenesis of IBD in chickens. Infection of chickens with IBDV was accompanied by the infiltration of CD4+ and CD8+ T cells in the bursa. There was an upregulation in the gene expression of important cytolytic molecules; perforin (PFN), granzyme-A, DNA repair and apoptotic proteins; high mobility proteins group and poly (ADP-ribose) polymerase in the BF. Importantly, PFN producing CD4+ and CD8+ T cells were also detected in the bursa of IBDV-infected chickens by immunohistochemistry. The IFN-Â³ expression was also strongly upregulated, suggesting the activation of T cells. The findings of this study will be useful for designing effective control strategies for IBDV based on vaccines that can augment T cell responses in addition to an antibody response.

Massively Parallel cDNA Sequencing (RNA-Seq) Analysis of Immune Tissues from IBDV-Infected Birds

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Massively parallel cDNA sequencing (RNA-seq) is a powerful tool for transcriptome analysis. In this study, commercial broilers were infected with the VarE strain of IBDV at 28 days of age. Seven days post-challenge, spleen, thymus, and bursa tissues were collected from both infected and control birds. Total RNA was purified and libraries were constructed for RNA-seq analysis. From these six libraries ~9 x 109 bp of sequence was aligned to the chicken genome. Sequences were assigned to 15,831 annotated genes (Gallus gallus Release 2.0) and expression frequencies were determined (cpmkb). For all three tissues, ~2,500 genetic elements were found to have greater than 2-fold changes in gene expression when compared to the appropriate tissue control. As expected, a very limited or repressed inflammatory response was observed in aLL of the tissues. In addition, genetic elements involved in the interferon response were found to be repressed in the bursa of infected birds. However, interferon gamma was found to be induced in the spleens of infected birds. Hierarchical clustering of the results further indicates that genes involved in cell division and DNA repair are induced in the spleen and bursa. Furthermore, as expected, genetic elements involved in apoptosis are induced in the bursa.

11086

Natural Reassortants of Very Virulent Infectious Bursal Disease Virus (vvIBDV) Containing Genetic Elements from Both Serotype 1 and 2 Viruses

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There are two serotypes of infectious bursal disease virus (IBDV) but only serotype 1 viruses are known to cause disease. The very virulent (vv) IBDV were identified in Europe during the 1980s and closely related genetic ancestors have spread around the world. In December 2008 the first vvIBDV were isolated in California. Subsequently, we isolated two viruses that appeared to be vvIBDV from a California layer flock in May 2009 and from a backyard flock of mixed breed chickens in October 2009. Nucleotide sequence analysis established their genome segment A was typical of vvIBDV but their genome segment B was related to serotype 2 IBDV. These reassortant viruses caused 20 - 60% mortality in specific-pathogen-free chicks and although they were infectious, no morbidity or mortality was observed in turkey poults. The apparent reassorting of California vvIBDV with an endemic serotype 2 virus indicates a common host and suggests vvIBDV may have entered California earlier than originally thought. The data reaffirm that molecular identification of vvIBDV from disease outbreaks requires sequence analysis of both genome segments.

11087

Combining FTA Card with Reverse Genetics Allows Characterization of the Antigenicity of Infectious Bursal Disease Viruses on a Global Scale

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Infectious bursal disease is a highly contagious and immunosuppressive disease in young chickens caused by infectious bursal disease virus (IBDV). IBDV is a double-stranded RNA virus able to infect and destroy proliferating B-lymphocytes which results in humoral immunosuppression. This increases the risk of diseases caused by facultative pathogens and failures of vaccinations. This virus is ubiquitously present in poultry flocks worldwide and is attempted to be controlled by vaccination programs. Due to the nature of RNA viruses able to quickly evade vaccination programs by selection of antigenic mutants by antigenic drift, determination of the antigenicity of circulating IBDV plays a critical role for the selection of efficient vaccines. The objective of our study was to determine the antigenicity of IBDV field strains at a global level using reverse genetics. To this end, we adapted the use of FTA cards for a safe transport of genetic material without being infectious and reverse genetics to determine the antigenicity of IBDV. By using a modified new protocol, RNA was isolated and amplified with specific primers encompassing the complete VP2 region responsible for the antigenicity of IBDV. The amplified cDNA was cloned into the IBDV reverse genetics system and analyzed for the nucleotide sequence and amino acid sequence. This was followed by reactivity with a panel of monoclonal antibodies to determine the antigenicity. By using this combined approach, IBDV can be antigenetically characterized for the first time from bursal samples across the globe without sending infectious material across country borders.

11088

Highly Pathogenic Avian Influenza H5N1 Natural Infection in Domestic and Free Living Water Fowls in Egypt

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Cloacal swabs were collected from domestic ducks and geese,and free living water fowls (Annas c.crecca, Gallinula c.choropus, swans, and Flulica arta arta). Four duck swabs and 2 from Flulica arta arta were positive on common A antigen and H5 rapid strips and direct ELISA, while were negative on H7 and H9 strips. Some of infected ducks showed nervous signs, clinical and post-mortem lesions were described. Swabs were confirmed positive in RT-PCR, then subjected to conventional PCR using specific four primers for full sequences of H5 gene. The amplification products were identified at the expected molecular weights.

Results are in accordance with the notion that several lineages of HA of HPAIV H5N1 strains are endemically co-circulating in Egypt. The sequences encoding the haemagglutinin (HA) of H5N1 isolates obtained in our work from different avian species in backyard holdings and poultry farms in Egypt revealed amino acid variations across the polypeptide and also in the polybasic cleavage motif of three of the isolates with one, so far, unique mutation in an isolate from a chicken.

11089

A Prime-Boost Approach for DNA-Mediated Vaccination against Infectious Bursal Disease in Broiler Chickens with Maternal Antibody

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Studies were carried out to determine whether protection of maternal antibody (Mab)-carrying broiler chickens by DNA vaccination against infectious bursal disease (IBD) could be enhanced by priming with an infectious bursal disease virus (IBDV) large segment protein-expressing plasmid and boosting with a killed IBD vaccine. One-day-old broiler chickens with Mab to IBDV were primed with various doses of DNA vaccine encoding large segment gene of IBDV strain variant E (VE) (P/VP243/E) and boosted with a single dose of killed IBD vaccine at 1 week or 2 weeks of age. Broiler chickens were challenged with IBDV strain VE at 3 weeks of age. Broiler chickens were not protected against IBDV challenge using the prime-boost approach when they were primed with less than 10 mg of P/VP243/E and boosted with a single dose of killed IBD vaccine. Broiler chickens primed with 10 mg of P/VP243/E and boosted with a single dose of killed IBD vaccine had 100 % protection against challenge by IBDV. Their bursa weight/body weight (B/B) ratios were significantly higher ($P<0.05$) than those in the challenge control (CC) group. The IBDV antigen was not detected in the broiler chickens protected against challenge by IBDV. Broiler chickens protected by priming with 10 mg of P/VP243/E had significantly lower ($P<0.05$) ELISA and virus neutralization (VN) titers to IBDV than those in the CC group after challenge. These results indicated that a prime-boost approach for DNA vaccination can provide protection of broiler chickens with maternally derived antibody against IBDV challenge by priming with a high dose of DNA carrying IBDV large segment gene and boosting with a single dose of killed IBD vaccine.

11090

Application of Statistical Text Analytics to Avian Influenza Scientific Literature

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There is a vast amount of international scientific literature on avian influenza. Text analytics (text mining) can be applied to current avian influenza research to help identify trends that can provide further insight to the scientific community. The goal of this project is to design a database based on a corpus of publications that is imported into a statistical software system to be processed, parsed and prepared for pattern recognition and statistical learning procedures. A preliminary dataset was utilized in the initial run of textual analytics using titles, author(s), abstracts and keyword(s) to cluster and indicate frequencies. In this paper, we will present the results of unsupervised clustering techniques that were used to categorize the documents into separate topic groups and supervised classification techniques to demonstrate how one might rapidly and efficiently categorize new documents into one or more important topic groups.

Breaks of H5N1 Avian Influenza in Previously Vaccinated Chicken Flocks

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A total of seven vaccinated layer and broiler-breeder chicken farms were monitored for H5 antibodies by HI and ELISA tests. Results revealed that the flocks possessed high levels of antibodies reaching 8 log2 by HI test and 1970 by ELISA test. Shortly after the last monitoring of antibodies, these flocks suffered from severe infection with avian influenza. Infection was proved positive by common A and H5 Rapid antigen capture immune-assay (AC-EIA). Tracheal swabs were confirmed positive in RT-PCR, then subjected to conventional PCR using specific four primers for full sequencing of H5 gene. Results are in accordance with the notion that several lineages of HA of H5N1 strains are endemically co-circulating in Egypt. The sequences encoding the haemagglutinin (H5) of H5N1 isolates obtained in our work revealed amino acid variations across the polypeptide and also in the polybasic cleavage motif of the isolates.

Sequencing of H5N1 Virus Circulating in Egypt

Salah Moussa and Omar Amen
Professor Emiritus

Use of high fidelity polymerase in sequence analysis demonstrated the HPAI cleavage site PQGERRRKKRGLF (single letter amino-acid notation) in all specimens. Our results were in accordance with the notion that several lineages of HA of HPAIV H5N1 strains are endemically co-circulating in Egypt. The sequences revealed amino acid variations across the polypeptide and also in the polybasic cleavage motif of the isolates with one, so far, unique mutation in an isolate from chicken in one amino acid at position 339 (G - E) within the HA cleavage site to the other Egyptian H5N1 genotypes without further changes of the multi basic HPAI characteristics. As an interesting case, one sample showed 2 virus populations during sequencing, which appeared as double peaks in electrograms, referring to possible super-infection of the infected bird which points to a probability of future mutation.

Salmonella spp, Escherichia coli and *Campylobacter spp* in Canadian chickens: updates on antimicrobial resistance trends, patterns and recovery rates

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The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors trends in antimicrobial resistance (AMR) in selected bacterial organisms for human, animal and food sources across Canada. The CIPARS components active in the chicken sector include abattoir, retail, and clinical/diagnostic. Important trends observed by CIPARS IN 2009 include: 1) Re-emergence of ceftiofur-resistant *Salmonella* and generic *Escherichia coli* from retail chicken meat samples and abattoir (i.e., cecal samples), and; 2) Trend towards increasing resistance to ciprofloxacin (CIP) in *Campylobacter spp* isolated from retail chicken meat samples. The CIP resistance appears to be regional (e.g., *British Columbia, Saskatchewan*) in distribution. Ceftiofur and drugs belonging to the fluoroquinolones (e.g., enrofloxacin, danofloxacin) are considered by the Veterinary Drug Directorate, Health Canada very important to human medicine. These drugs are not registered for use in chickens in Canada. Antimicrobial use (AMU) data from this sector is currently unavailable but a national AMR/AMU framework has been developed by the CIPARS Farm Working Group in consultation with the poultry industry and is now being piloted in broiler farms across Ontario beginning in the summer of 2010. Recovery rates for the different enteric organisms monitored by CIPARS are as follows: *Salmonella* in abattoir samples-27%; *Salmonella* in retail meat samples – 39 to 48%, *Campylobacter* in retail meat samples – 20 to 53%. Antimicrobial resistance trends of selected antimicrobials from 2003 to 2009 and AMR patterns will also be described.

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Characterization of Ornithobacterium Rhinotracheale From Commercial Chicken and Turkey Flocks in Russia

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A total of 19 Ornithobacterium rhinotracheale (ORT) isolates were recovered from chickens and turkeys in Russia. All isolates were able to agglutinate chicken and sheep erythrocytes irrespective on colony morphology. Most of the ORT isolates were sensitive to commonly used antimicrobial agents applied such as cefoperazone, levomycetin, ampicillin, baytril, furadonin. However, resistance to amikacin, benzylpenicillin, gentamicin, clindamycin, norfloxacin, erythromycin and trimethoprim-sulfamethoxazole was also observed. The 16S ribosomal RNA (rRNA) sequences of ORT isolates demonstrated extremely high identity (99%100%) among themselves and to sequences from GenBank

11095

Prevalence of Salmonella, Campylobacter and E. coli in Wild Game Birds 2009/2010 Season

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Wild bird hunting is a significant part of life in the rural Midwest; however the potential exposure of humans to pathogens through field dressing and the consumption of wild game birds are relatively unknown. This study assessed the prevalence of foodborne pathogens such as Salmonella, Campylobacter and E. coli in wild birds hunted in the ND/MN region. Samples of gut of field dressed birds included pheasants, grouse, partridge, goose and wild ducks. All gut samples were tested for the presence of pathogens using standard methods and isolates recovered were characterized for antimicrobial susceptibility and virulence using molecular analysis. Salmonella was not detected in any birds tested; antimicrobial resistance in E. coli was relatively low suggesting limited exposure of wild birds to antimicrobial agents; the majority of isolates did not appear to harbor genes associated with pathogenesis. Campylobacter was recovered from a considerable number of samples with most isolates identified as C. jejuni. Further analysis to assess the pathogenesis of Campylobacter strains is ongoing to determine the potential risk of exposure through the consumption of game birds.

11096

Biological Monitoring of Vaccine Take and Productive Parameters in Broilers Vaccinated with Immune Complex and Recombinant Vector Vaccines against Infectious Bursal Disease

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Two recent scientific concepts of vaccines against IBD, a live Immune Complex vaccine and a recombinant vector vaccine were compared for their biological characteristics of vaccine “take” and productive parameters from vaccinated broilers. The immune complex vaccine is composed of an IBD vaccine strain virus (Winterfield 2512) artificially complexed with specific antibodies against it and the recombinant vector vaccine possesses a recombinant HVT strain of Marek’s virus expressing the VP2 protein gene from a classical strain of the IBD virus (IBDV). Two (2) controlled field trials in low IBDV field challenge farms were carried out to monitor the expected biological characteristics of vaccine “take” between 35 and 42 days of age (IBD serology / Bursa of Fabricius [BF] weight / lymphoid depletion / BF viral colonization by molecular techniques). In addition, productive parameters of broilers vaccinated with both vaccines were also compared (average slaughter weight, feed conversion, total mortality, daily weight gain). Both vaccines were applied via subcutaneous route at 1 day of age in both trials to a total of 316,080 broilers.

Evaluation and Validation Studies of Real-time PCR Assay for the Detection of Chlamydophila Psittaci

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Chlamydophila psittaci is a lethal intracellular bacterial species that causes endemic avian chlamydiosis, epizootic outbreaks in mammals, and respiratory psittacosis in humans. Chlamydia isolation in cell cultures and embryonating chicken eggs are routinely conducted at Penn State Animal Diagnostic Laboratory. The cell culture and chicken embryo materials were used for chlamydia detection by fluorescence antibody (FA) test. Because of the availibility of specific and sensetive chlamidia FA conjugate, a real-time polymerase chain reaction (rtPCR) assay for the detection of chlamydophila psittaci has been recently studied in comparison with chlamydia isolation and FA test. The rtPCR assay was performed on tissue homogenate, chicken embryo and cell culture samples of FA confirmed positives and negatives. Preliminary results show good correlation and agreement of the chlamydia rtPCR and FA test and chlamydia isolation. This comparison study will be continued for more data collectionso as to fully validate the rtPCR for the detection of chlamydophila psittaci from clinical and fileld specimens without isolation procedures. The chlamydia rtPCR primers are designed as general primers to chlamydophila psittaci to detect all genotypes.

Aspergillus fumigatus in Day-Old Broiler Chicks: What Does It Mean?

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It is not uncommon for broiler production companies in the southeastern United States to periodically check newly hatched broiler chicks for bacterial and fungal infections. Of particular interest are those companies that frequently find a high prevalence of *Aspergillus fumigatus* in those day-old chicks. As might be expected, these companies may have some broiler flocks that develop the clinical disease of aspergillosis. However, there are numerous times that flocks represented by *Aspergillus fumigatus* positive day-old chicks experience normal seven-day mortality and never develop the clinical disease of aspergillosis. This raises a question about the importance of finding *Aspergillus fumigatus* in chicks at day one. For this study, two flocks of broilers were sampled at one day of age and identified as culture positive for *Aspergillus fumigatus*. These flocks were sampled again at 7-, 14- and 21-days-of-age. All culture and histopathology results will be presented.

11099

Macrorhabdus Ornithogaster (Megabacterium) Infection in Adult Hobby Chickens in the North Georgia Mountains, USA

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A field investigation was conducted on a flock of adult hobby chickens showing intermittent signs of enteritis. A rooster examined in the initial field visit and post-mortem had cecal worms, roundworms, trichomoniasis, coccidiosis, and Macrorhabdus ornithogaster was diagnosed histologically in the mucosal isthmus of the proventriculus and ventriculus. Three roosters and two hens were examined in a follow-up investigation of the flock nine days later. Macrorhabdus ornithogaster was confirmed in one hen.

11100

Pathogenicity and Molecular Detection of Chicken Anemia Virus in Commercial Broiler Farms in Venezuela

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This investigation was performed in a sample of 320 broilers chickens in growth around 1 and 4 weeks old, they presented unspecific clinical signs. The necropsy revealed bone marrow from rosy to yellowish-whitish, thymus atrophy and pale livers. Histopathology revealed thymic cortical lymphoid depletion from mild to severe, anisocytosis and anisokaryosis. Eosinophilic intra-nuclear inclusion bodies were detected in lymphocytes and hyperplasic reticular cell also showed intranuclear eosinophilic inclusion bodies. In the bone marrow, hypoplasia from mild to severe with depletion of the red blood cell lineage as well as the granulocytic lineage accompanied by replacement with adipocytes. Foci of hyperplasia with erythropoietic and myelopoietic intense activity, some stem cells were hypertrophic, with large nucleus and small inclusion bodies were observed. The hepatic lesions were characterized by the presence of perivascular infiltration of mononuclear cells, fat degeneration, hepatocellular necrosis and biliary hyperplasia, in some cases there were intranuclear eosinophilic inclusion bodies consistent with adenovirus in hepatocytes. The PCR showed the presence of CAV DNA in 19 of 40 samples, in 9 of 10 farms evaluated. The Pearson Correlation analysis confirmed the presence of an association between the variables thymus atrophy and bone marrow lesions (r= 0.3642; P=0.0209) a characteristic of the CAV. The Sparkman Rank analysis demonstrated that the bone marrow lesions are connected narrowly to the CAV diagnosed using PCR (r= 0.7165; P= 0.0000). The results of histopathology, PCR and gross lesions confirmed the diagnosis of CAV in the affect farms and demonstrated its virulence in commercial broilers.

Alternative Methods for Detection of Chicken Infectious Anemia Virus (CIAV)

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CIAV is a widely distributed immunosuppressive agent. Specified pathogen free (SPF) flocks and eggs used for vaccine production and diagnostics must be CIAV-free. Detection of CIAV infection in SPF flocks involves primarily serology or other invasive methods. In an attempt to develop alternative non-invasive tools for rapid detection of CIAV infection, a trial was conducted in serologically-negative broiler breeder pullets vaccinated with a commercial live-attenuated CIAV vaccine. Controls and vaccinated groups were sampled at different time pre and post vaccination. Conventional and non-conventional samples were used for CIAV DNA detection. Chicken serum was used for antibody detection using ELISA.

A case of unilateral periorbital cellulitis and mandibular osteomyelitis in a turkey flock

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A farm of meat turkeys in France was affected by a condition characterized clinically by a severe inflammation of the orbital region and progressive crossing of the beaks, observed for 3 successive batches in 2010. The clinical outbreaks affected turkeys aged from 3 to 12 weeks, with a daily incidence of 1/1000 new cases. The two poultry houses of the farm were concerned in the same way and epidemiological investigations could exclude obvious toxic, genetic, technical or nutritional causes.

Affected turkeys first expressed unilateral swelling of the orbital region, followed few days later by a lateral deviation of the upper part of the beak. Birds stayed in very good general condition and could survive days until the deviation of the beak stopped them from eating. Beak and bones were still hard and non flexible. At necropsy, an increased size of the affected eye could be reported, mainly associated with an heterophilic exudate behind it. In he most severely affected birds, necrosis and complete loss of the temporomandibular joint could be observed. No other gross lesion could be observed.

Staphylococcus aureus was systematically isolated and characterized. Histopathological examination of several tissues including eyes, jaw bones and respiratory tract were extensively processed. A severe osteomyelitis was systematically observed. The complete histopathological picture is presented and discussed.

To our knowledge, it is the first case report of an orbital cellulitis, with crossed beaks, affecting turkeys.

11103

Quantitative Analytical Technique Applied to Histopathology of Birds Infected Experimentally by Chicken Anemia Virus

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Agroservices Luzvill, C.a.

Five slides from healthy age matched chickens controls and five slides from chickens experimentally infected with chicken anemia virus between one and twenty one days post infection (PI), were analyzed at magnifications of 200X and 400X. Histopathology showed severe bone marrow hypoplasia to complete aplasia, full depletion of the erythrocytic and granulocytic series, both accompanied by space occupying adipocyte replacement. Foci of erythropoietic hyperplasia with intense myelopoietic activity, and some hemocytoblast hypertrophy were present in the bone marrow. A quantitative analytical technique by Positive Pixel Count Algorithm was applied. It demonstrated that measured areas stained of control slides were higher than CAV slides (Average: 61% vs. 25%, respectively). The control slides showed strong positivity, due to the presence of a larger quantity of erythrocytic and granulocytic cell series. The CAV slides at seven days PI had high positivity (average: 94%), it was explained because the chicken anemia virus takes time to replicate and severe lesion do not appear until 10 to 17 days PI. After 21 days PI the cellular regeneration is observed by means of foci of erythroblastoid cell hyperplasia. This technique demonstrates in a quantitative way the severe decrease of the cellular components in the bone marrow with infection of CAV, supporting with numeric data the histology image evaluated by the pathologist. Therefore, it can be used as support to the histopathology of field samples to evaluate the presence of lesions caused by CAV and this way to improve the quality and efficiency of the veterinary pathology services.

11104

Enteritis and Elevated Mortality in Young Guinea Fowl

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Meat-type guinea fowl on a commercial, multi-age farm exhibited enteritis and increased mortality that peaked at day 12-14 in affected flocks. Cumulative mortalities reached 36.4% at 21 days. Affected keets were small, lethargic, anorexic, and dehydrated. Thymic atrophy, necrotic cores in the bursa of Fabricius, and mild duodenal edema were present on gross examination. Histopathology and fecal floatation revealed coccidiosis. Rotavirus and astrovirus were identified by PCR from the feces; no Salmonella spp. were isolated. Although weekly flock mortalities returned to acceptable limits, poor flock uniformity and performance was evident throughout the life of the flock.

11105

Investigations about the Etiology of Runting Stunting Syndrome Affected Chickens by In Situ Hybridization

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Runting and Stunting Syndrome (RSS) is an enteric disease that causes severe weight suppression during the first few weeks of age and is associated with lack of flock uniformity. Cystic enteropathy is a hallmark lesion observed in the small intestine by microscopical examination. The disease was reliably reproduced using filtered intestinal homogenates from RSS affected broilers implying a viral etiology. Based on subtractive metagenomics studies four viruses were identified which might play a role in the etiology of RSS. The genetic information of three different chicken astroviruses (CkAstv) and a chicken parvovirus (CkParv) was cloned and used as negative-sense riboprobes for in situ hybridization (ISH). One day old commercial broiler chickens were exposed to RSS-contaminated litter and one group was placed on fresh wood shavings. Five chickens were obtained at each day from day 1 to 5 p.i. and formalin-fixed paraffin-embedded tissues of the duodenal loop were analyzed by two approaches: ISH and microscopical examination. Nucleic acids of two CkAstv were identified only in the villous epithelial cells. Interestingly the nucleic acid of the third CkAstv was observed in both, the crypt as well as villous epithelial cells. No ISH signal was observed using the CkParv probe. Interestingly, cystic enteropathy and ISH signals were progressive and increased with age but not always associated with each other. The presence of ISH signals in the crypt region of the duodenum strongly suggests that a new CkAstv is involved in the etiology of RSS.

11106

Characterization of a Novel Chicken Astrovirus Isolated from Intestinal Homogenates of RSS-Affected Chickens

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Runting Stunting Syndrome (RSS) has been an important disease in the broiler industry since its first report in 1970s. Isolation of responsible pathogen candidates is fundamental to understand the pathogenesis of the disease and for vaccine development. Although some viruses have been suggested as pathogens, no viruses have been identified as responsible pathogens. Recently, a new chicken astrovirus (CkAstv) was proposed as possible RSS pathogens (Sellers, et al. Vaccine 28:1253-1263, 2010). In isolate a virus which might play a role in RSS filtered intestinal homogenate of RSS-affected chickens was passaged on different cell lines. Using a polyclonal antisera specific for the capsid protein of the new CkAstv specific immunofluorescence was observed in LMH cells. Exclusion of presence of other viruses that might be related to RSS was conducted by PCR and RT-PCR and serological methods. In addition, the isolate is antigenically different from the known CkAstv 1 and CkAstv 2 as indicated by absence of a specific signals by indirect immunofluorescence IFA and Western blot. The complete genome sequence of new CkAstv was determined and showed an unusual genomic organization for Astroviridae. ORF1a and ORF1b are encoded by two separate open reading frames without an indication of a frame shift site. The most similar virus was the duck astrovirus for the ORF1a encoded protein (56% identity), the turkey astrovirus 2 for the ORF1b encoded protein (72%), and the duck astrovirus for the capsid protein (36%). The obtained results clearly indicate a novel CkAstv from RSS affected chicken.

11107

Efficacy of BMD Versus Probiotics in the Feed for the Control of Necrotic Enteritis by Clostridium Perfringens in Broiler Chickens

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This study was conducted to obtain data on the effects of feeding BMD (bacitracin methylene disalicylate) alone and in conjunction with six probiotics to birds receiving a Clostridium perfingens challenge. BMD was used at 50g/ton. The NE challenge (positive controls) resulted in significant mortality (22.16%) and lesions. BMD treatments alone or in combination with the probiotics significantly lowered the NE mortality and NE lesion scores compared to the challenged controls

11108

Role Of Maternal Antibodies in Protection Against Chicken Parvovirus-Induced Runting-Stunting Syndrome

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PCR-based nation wide survey in the USA indicated a wide prevalence of chicken parvovirus (ChPV) in poultry flocks. In addition, experimentally infected broiler chickens showed characteristic runting-stunting syndrome (RSS) following ChPV inoculation indicating a major role of ChPV in the etiology of enteric diseases in chickens.

To determine the effect of parvovirus specific antibodies on the development of enteric disease, Mab positive and Mab negative day-old broiler chickens were infected orally with 10e6 ChPV particle and protection was evaluated. Typical clinical signs of RSS were observed in Mab free birds characterized by diarrhea and 40, 21 and 17% growth retardation at 7, 14 and 28 days post inoculation (DPI), consequently. Parvovirus-infected maternal antibody positive chickens showed significantly lower growth retardation, 23 to 13% between 7 and 14 DPI ($p<0.001$) when their weight gains were compared to those observed in non-infected control birds.

In order to study the enhancement of immune response of layers to parvovirus, experimental inoculations were performed with inactivated ChPV and/or baculovirus recombinants, which expressed the ChPV VP2 antigen. Three-week-old SPF chickens showed positive antibody response following immunization with inactivated parvovirus and exhibited significantly higher serum ELISA antibody titers when they were boost-inoculated with BV-VP2 recombinant.

These data strongly suggest that immunization with inactivated parvovirus alone or in combination with VP2 antigen will induce high-level immune response in layers and subsequently the maternally derived antibodies may provide full protection in young chickens against parvovirus induced enteric disease.

11109

Comparison of Incidence of Poultry Diseases in Commercial and Noncommercial Poultry in North Alabama

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State Of Alabama Department Of Agriculture And Industries

Veterinary diagnostic laboratories serve an integral function to industry through surveillance and monitoring for reportable diseases. An often time overlooked benefit of the diagnostic laboratory is the utilization of results to establish trends and identify threats from diseases that are not reportable, yet have economic bearing on the commercial and non-commercial producer alike. In this poster a graphic summary of disease occurrence over the last two years in both commercial and noncommercial poultry in North Alabama is presented in order to identify possible threats by each sector.

11110

Characterization and Distribution of Avian Pathogenic Escherichia Ccoli Isolates from Broilers in Peru

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Technical Department Of Innova Andina S.a., Lima, Peru

Avian colibacillosis produced due to Escherichia coli infections, is cause of significative mortality and morbility in poultry households. Previous research has identified virulence factors commonly associated with avian colibacillosis. The objective of this study was to identify by MULTIPLEX PCR the pathogenic isolates present in peruvian broiler farms, based in the presence of 5 genes codifiers of virulence factors: iss (increase serum survival), iucC (aerobactin iron sequesterin system), tsh (temperature-sensitive hemagglutinin), cvaC (operon colicin ColV) and irp2 (iron repressible protein). A total of 36 poults between 14 and 31 days old from 3 different poultry farms located in the north, center and south of Peru were sampled, obtaining 3 suspected E. coli isolates per bird (108 colonies in total). Each sample was biochemically tested with TSI, SIM, LIA, CITRATO and EMB mediums for the confirmation of the diagnosis. The colonies that resulted positive (37.96 % of the isolates) were analyzed by MULTIPLEX PCR, obtaining that 65,85% of them were classified as pathogenic (carriers of two or more virulence genes). This results show a strong presence of potentially pathogenic E. coli strains in Peruvian broiler households.
Key words: Escherichia coli, pathogenic, genes, virulence, broilers, Peru

11111

Serum Survival in Avian Pathogenic Escherichia Coli

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Serum resistance has been recognized as an important trait in the pathogenesis of avian pathogenic Escherichia coli (APEC), the causative agent of colibacillosis. Despite its importance, the underlying means by which APEC resist the bactericidal effects of host serum is poorly understood. Although the roles of a smooth lipopolysaccharide layer, certain types of capsule, and the traT, ompA and iss outer membrane proteins in serum resistance of some E. coli has been documented, transcriptome analysis of APEC, when grown in serum, showed no evidence of their involvement. However, other putative mechanisms of serum resistance were identified that make explain APEC's ability to survive in the bloodstream to cause colisepticemia.

11112

Characterization of Attaching and Effacing Escherichia Coli Isolated From Poultry

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Attaching and effacing Escherichia coli (AEEC) are characterized by their ability to cause attaching-and-effacing (A/E) lesions in the gut mucosa leading to diarrhea. Although AEEC strains from other animal species such as bovine and porcine have been extensively characterized, only a limited number of studies have been undertaken to characterize AEEC of avian origin. The present study was conducted to better characterize AEEC strains isolated from poultry. A total of 50 AEEC strains isolated from chickens and turkeys with diarrhea were analyzed to characterize their O serogroups, antimicrobial susceptibility patterns, virulence gene profiles, plasmid profiles, phylogenetic groups and multilocus sequence types (MLST). Further, AEEC strains were subjected to a PCR-restriction fragment length polymorphism analysis (RFLP) assay to identify allelic variants of their eae locus.

11114

Pathogenicity of Colombian Infectious Bronchitis Virus Isolates

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A recent study, Characterization of infectious bronchitis virus (IBV) field strains from Colombian poultry during 2009 presented at 2010 International Poultry Scientific Forum in The Southern Conference on Avian Diseases 51st Annual Meeting, described that IBV isolated in Colombian poultry were related with vaccine and field strains.

The aim of this study was to evaluate the pathogenicity of three IBV Colombian isolates of different geographical origin, one genetically related to vaccine strain and the others considered field strains. Sixty three broilers free of IBV antibody specific were divided in groups of 21 birds and housed in three pens. Groups were inoculated ocularlly and intratracheally at 28 days old with 200ul 1x103.5 ELD50 of each virus. Clinical signs were observed and scored. Five days post-inoculation seven broilers of each group were euthanized and samples of tracheal swabs, conjunctiva, trachea, cecal tonsils, kidney, blood and cloacal swabs were collected to virus isolation, PCR, histopatology and serology. In all the groups birds showed respiratory signs. PCR were positive from all samples collected. Virus isolation and histopatology are in process. Variations in severity of clinical signs were observed and virus was detected in different organs among groups.

11115

Host Driven Selection of Infectious Bronchitis Virus

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Each infectious Bronchitis Virus (IBV) isolate exists as a genetically diverse population of virus particles. To analyze host driven selection on that population, we infected one SPF chick with the Arkansas DPI strain of IBV and allowed it to be naturally transmitted ten times to contact birds. The transmitted virus was then passaged in embryonated eggs ten times. At each transmission in chickens we genetically characterized the virus, measured the viral load, infectious period, latent period, and clinical signs. At each passage in embryonated eggs, we genetically characterized and measured the titer of the virus. Implications of host driven selection on the emergence of variant viruses will be discussed.

11116

Phylogenetic Analysis and Identification of Infectious Bronchitis Virus Strains Worldwide

Mark W. Jackwood, Deborah A. Hilt
University Of Georgia

Many IBV spike sequences in the GenBank database are similar to previously recognized and well-characterized strains, particularly vaccine type viruses, but are not identified as such. Often, sequences of newly isolated viruses show close relationships with presumably unknown or unique IBV isolates in the database leaving unanswered questions regarding the actual type of the virus. In this study, spike sequences from IBV isolates worldwide are analyzed using bioinformatics to determine their groupings with previously identified and characterized strains of the virus. This information is needed to establish if recently isolated viruses are similar to known IBV strains or are new variant viruses.

11117

Correlation Between Phylogenetic Topology and Antigenic Properties Determined by Virus Neutralization of Field Infectious Bursal Disease Viruses Detected in US During 2009 and 2010

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During years 2009 and 2010, sixty-seven infectious bursal disease viruses from four different Southern U.S. states were detected by RT-PCR from bursal tissue samples collected from commercial broiler flocks. The sequence analysis of the hypervariable region of the VP2 gene showed that all the viruses were genetically similar to Delaware E, with genetic similarities between 95.9% to 97.5% in their nucleotide sequences. The antigenic index profiles of these field viruses determined by the Jameson and Wolf method were similar to that observed with Delaware E. However, the phylogenetic analysis by Neighbor Joining method grouped these IBD viruses into two different clusters. Six viruses that exhibited the highest phylogenetic distance to Delaware E were adapted to grow in SPF chicken embryonated eggs to conduct virus neutralization tests. The 1084E variant and the Lukert strains were used as references. The relationships between the phylogenetic analysis and the results of neutralization tests are discussed. Preliminary data generated in our laboratory suggests that these nucleotide differences do not have a significant effect on the neutralization activities of polyclonal antibodies.

11118

Molecular Diversity in the Hypervariable Region of VP2 from Infectious Bursal Disease Viruses

Daral J. Jackwood and Susan E. Sommer-Wagner
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There are two antigenic serotypes (1 and 2) of Infectious bursal disease virus (IBDV). The serotype 1 viruses are generally divided into two antigenic sub-groups; classic and variant strains. Although this phenotypic picture seems relatively simple, molecular data suggest IBDV antigenicity may be much more diverse. Amino acids at key positions on the hydrophilic loop structures of the hypervariable region of VP2 (hvVP2) control antigenicity and can vary extensively among viruses. Variant viruses are typically 222T, 249K, 286I and 318D while classic viruses are 222P, 249Q, 286T and 318G. We have identified viruses with a combination of amino acids at key positions that suggest their antigenicity would be a hybrid of the variant and classic types. These molecular data combined with published but less extensive *in vivo* antigenicity studies suggest IBDV may be much more antigenically diverse than the commonly used classic and variant designations.

Reassortant Infectious Bursal Disease Viruses from California with a vvIBDV Genome Segment A and a Serotype 1 NonvvIBDV Segment B

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Infectious bursal disease virus (IBDV) infection in immature chickens induces immune suppression by the destruction of B- lymphocytes in the bursa of Fabricius. The very virulent pathotype of IBDV (vvIBDV) causes high mortality and was first discovered in Belgium in the 1980 s. The vvIBDV have spread throughout Europe and have since been reported on most continents including Asia, Africa, Central, South and North America. IBDV is a non-enveloped, double-stranded RNA virus. The multi-segmented nature of the genome facilitates reassortment of the genome segments. Since the first confirmed diagnosis of vvIBDV in California poultry in December 2008, several reassortants with a genome segment A matching the very virulent pathotype and segment B matching non-vvIBDV strains have been diagnosed in commercial and backyard poultry flocks. The phylogenetic relationship of both segments A and B with other published IBDV sequences as well as the pathogenicity of a California isolate with a vvIBDV genome segment A and serotype 1 non-vvIBDV genome segment B was investigated in immunologically naïve chickens and was compared with other classic, variant and vvIBDV isolated from poultry in the USA.

11121

Development of a Molecular Typing Method for Enterococcus cecorum

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D. S. Wijetunge*, J. Blair, P. Dunn, E. Wallner-Pendleton, V. Lintner, and S. Kariyawasam Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802

Enterococcus cecorum is an emerging pathogen of poultry, in particular of male broiler and broiler breeder chickens. It has been implicated in septicemia, and progressive lameness and paralysis due to joint and bone infections. Economic losses from the disease are attributed to increased mortality and culling rates, decreased average processing weights and increased feed conversion ratios. Tracing the source of an outbreak is crucial to implement effective control strategies. The objective of this study was to develop a DNA fingerprinting method to type E. cecorum. We evaluated three typing methods, namely, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) analysis and enterobacterial repetitive intergenic consensus (ERIC) PCR analysis for typing 25 isolates of E. cecorum strains isolated from clinical specimens and other sources. This presentation describes the suitability and discriminatory power of each typing method as a DNA fingerprinting tool for E. cecorum.

11122

Genotypic and Phenotypic Comparison of Field Isolates of Enterococcus cecorum in Outbreaks of Spondylitis

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Enterococcal spondylitis, due to Enterococcus cecorum, is an emerging disease now predominately afflicting broiler males in the United States causing lameness, paresis, and/or paralysis. Morbidity can reach 35% and mortality 15% in some flocks. Field isolates of E. cecorum collected from spinal abscesses and gastrointestinal tracts were analyzed by the Omnilog® Identification System from Biolog, Inc. and with Sensititre® Avian Plates allowing for phenotypic comparison of strains. A subset of isolates, representing all farms investigated, was analyzed by pulsed field gel electrophoresis permitting genotypic analysis. Phenotypic and genotypic data will be compared to analyze the pathogenicity of E. cecorum isolates.

11124

An Evaluation of Optimal Methods for Avian Influenza Virus Sample Collection

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Sample collection and transport are critical components of any diagnostic testing program and due to the amount of avian influenza virus (AIV) testing in the US and worldwide, small improvements in sensitivity and specificity can translate into substantial cost savings from better test accuracy. Also, details on optimal testing can provide valuable information for developing optimal testing programs for different compartments and situations. Some elements of AIV sample collection that we evaluated are swab type and transport conditions. New technological advances in swab technology have show improvements in test sensitivity for seasonal influenza testing. Therefore we evaluated the performance of urethane foam, nylon flocked swabs, and standard Dacron swabs for the detection of low pathogenicity AIV from oral and cloacal swabs from experimentally infected chickens by virus isolation, real-time RT-PCR and commercial antigen detection assay. We also evaluated the effect of different transport conditions, wet in media or dry in a culturette, on virus detection and recovery from chickens experimentally infected with AIV.

New Approaches to Develop Improved Molecular Diagnostic Essays for Infectious Diseases [Essay Design]

Hayet Abbassi
Department of Animal Science and Department of Population Medicine,
University of Minnesota, St. Paul, MN 55108

Molecular diagnostic tests are considered as simple, mature and “gold standard” tools while they are in reality complex, evolving and variable.
During an investigation performed in the molecular diagnostic development section of the Minnesota Veterinary Diagnostic Laboratory (MVDL) aimed originally to improve the molecular diagnostic test for avian Metapneumovirus (aMPV), while leading and performing the project titled “Improvement of TaqMan RT-PCR for Avian Pneumovirus Through Nucleocapsid Gene Targeting”, I had the chance to participate in other molecular development projects on various infectious agents and also to participate in the daily molecular diagnostic operations at their various stages from sample processing to result reporting. This multifaceted experience allowed me to develop new approaches for either developing a new molecular assay or improving the sensitivity, specificity and efficiency of the existing assays. The strategies I retained are simple but key steps and criteria that are necessary for achieving a good molecular essay. Most of these strategies were incorporated in the molecular testing of the MVDL and allowed to improve several molecular essays for various pathogens. The first part of this paper will focus on the introduction and the different aspects of the essay design and the second part of the paper will focus on the different aspect of the optimizations and validation of the molecular essays and the results of the improved aMPV molecular test.

New Approaches to Develop Improved Molecular Diagnostic Essays for Infectious Diseases [Optimization and Validation]

Hayet Abbassi
Department of Animal Science and Department of Population Medicine,
University of Minnesota, St. Paul, MN 55108

Molecular diagnostic tests are considered as simple, mature and “gold standard” tools while they are in reality complex, evolving and variable.
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Fowl Cholera Vaccination of Pen-Raised Ring-Necked Pheasants with Commercially Available Vaccines

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Fowl cholera is an occasional disease of pen-raised ring-necked pheasants that results in high morbidity, excessive mortality, and chronic poor performance. Two commercially available Fowl cholera vaccines were administered to four replications of 100 ten week old ring-necked pheasants and observed for four weeks for changes in livability and weight gain as compared to controls. At four weeks post-vaccination, 25 pheasants were inoculated with 4000 cfu of a previously isolated pheasant Pasteurella multocida and were observed for four weeks for changes in livability and weight gain as compared to controls. Mortality and weight gain was negatively influenced following vaccine administration for one of the vaccines. Livability and weight gain after challenge was reduced compared to sham inoculated controls but significantly improved over non-vaccinated challenged controls for both vaccines. Commercial vaccination may provide some protection for ring-necked pheasants against Pasteurella multocida challenge.

11128

Retrospective study of Novel Picornavirus associated with Turkey Viral Hepatitis

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Turkey viral hepatitis (TVH) was first reported in 1959 by Snoeyenbos et al. in the United States and Mongeau et al. in Canada. The disease is characterized by white foci of necrosis in the liver and occasionally in the pancreas. The cause has not been established, even though, the disease has been known for more than 50 years ago. However, recently a novel Picornavirus was identified as the probable cause of TVH, based on pyrosequencing.

The relative frequency of TVH between 1989 and 2010 will be determined by retrieval of data diagnosed as TVH. The age of the turkeys, clinical signs, mortality pattern, seasonality and its occurrence in various ranches will be determined from the data. Ten cases have been selected for detailed study such as determination of the virus in the sera, liver, pancreas and feces by PCR. Insitu hybridization and immunohistochemistry will be performed on the liver, pancreas, spleen, intestine and bursa of Fabricius of all birds and heart, kidneys and lung of a few birds to study the distribution of the virus. Preliminary results suggest that the virus affects not only the liver and pancreas but also the intestine of poults. Finding picornavirus in the intestine is noteworthy as it adds another virus to the list of viruses associated with poult enteritis. Data will be collated on the epidemiology of TVH and the presence of virus in various organs and the results will be presented and discussed.

11129

Detection of Lymphoid Leukosis Tumors in White Leghorn Chickens of Line alv6 that is Resistant to Subgroups A and E Avian Leukosis Virus and Maintained Under Specific Pathogen-Free Conditions

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Chickens from Avian Disease and Oncology Laboratory (ADOL) line alv6 that is known to be resistant to infection with subgroups A and E avian leukosis virus (ALV) were vaccinated at hatch with a Marek s disease (MD) vaccine containing serotypes 1, 2 and 3 MD viruses, and were maintained under specific-pathogen free (SPF) conditions from the day of hatch until 56 weeks of age. Lymphoid leukosis tumors were detected in several chickens that died after 20 weeks of age. Chickens tested negative for all subgroups of exogenous ALV and for antibodies against ALV of subgroup of A, B and J. Also, tumor tissues tested negative by PCR for the presence of infectious ALV of subgroups A, E, and J. Results suggest the development of spontaneous LL in SPF white leghorn chickens that are resistant to subgroup A and E ALV. The role of using MD vaccines containing all serotypes of MD virus in the development of these tumors will be discussed.

Current Status of the National Poultry Improvement Plan

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The National Poultry Improvement Plan celebrated its 75th year of existence in 2010 by holding the 40th Biennial Conference in San Diego, CA. The success of the Plan has been largely due to its ability to be modified relatively quickly with the cooperation of industry and state representatives. Thirty-seven proposals were brought forth for consideration at the Biennial Conference including a very important SE program for broiler breeders. Results of delegate voting on these proposals and the status of the Plan in general will be discussed.

11131

Significant Poultry Disease Notifications in Arkansas, Missouri, and Oklahoma: A Model Voluntary System

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The Poultry Federation Lab

There are statutory requirements to notify officials of the diagnosis of certain animal diseases, however, laws are not clear on case definition or notification methods. State Boards have the authority to discipline veterinarians for failure to report diseases, but not all states have penalties for laypersons who know of, but do not report cases of these program diseases. Also, there are discrepancies in the methods used by state officials to notify stakeholders of situations that may increase risks to a disease-free flock. Similarly, some states require the reporting of disease detection for certain endemic pathogens. Because of the use of vaccines, as well as complacency regarding certain common disease situations, it is likely that some diseases are detected more often than they are reported to state officials. Other than compliance with statute, there is little benefit to applying additional resources to foster reporting of many endemic diseases.

The Poultry Federation is a non-profit trade organization that represents the poultry and egg industries in Arkansas, Missouri, and Oklahoma. Most poultry producers in these states participate in a voluntary stakeholder disease reporting system that is managed by The Poultry Federation. This system has been successful in covering some of the gaps in the statutory reporting systems. However, some stakeholders continue to express frustration about issues created by a dual reporting system. This comentary describes the steps for reporting a poultry disease diagnosis in Arkansas, Missouri, and Oklahoma. Special consideration is given to ambiguous reporting expectations or variable compliance practices. A comparison with some other disease reporting systems is included.

11132

The Council for Agricultural Science and Technology: A Vital Poultry Industry Partner

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The Council for Agricultural Science and Technology (CAST) is a nonprofit 501 (c)(3) organization composed of scientific societies and many individual, student, company, nonprofit, and associate society members. CAST's new structure consists of a Board of Representatives (composed of representatives of the scientific societies, commercial companies, and nonprofit or trade organizations), a Board of Directors (formerly the Executive Committee), and a Board of Trustees (formerly the National Concerns Committee). The primary work of CAST is the publication of task force reports, commentary papers, special publications, and issue papers written by scientists from many disciplines. These publications and their distribution are fundamental activities that accomplish CAST's mission to assemble, interpret, and communicate credible science-based information regionally, nationally, and internationally to legislators, regulators, policymakers, the media, the private sector, and the public. The wide distribution of CAST publications to nonscientists enhances the education and understanding of the general public. CAST has earned a solid reputation for the fair, unbiased exchange of agricultural information over the past 38 years. Membership is composed of professional scientific societies (including AAAP, AVMA, and PSA), sustaining companies and nonprofits, subscribers, and individuals. Recent poultry-related publications include two commentaries: "Avian Influenza: Human Pandemic Concerns" and "Avian Influenza: Trade Issues", and two issue papers: "Poultry Carcass Disposal Options for Routine and Catastrophic Mortality" and "Vaccine Development Using Recombinant DNA Technology". These papers involved some AAAP members as authors or reviewers. AAAP representatives to CAST continue to play an active role in promoting CAST's mission.

11133

NEW INSIGHTS INTO PLASMID-ASSOCIATED PHENOTYPES AND GENOTYPES OF APEC STRAIN CHI7122 (O78:K80:H9)

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Extra-intestinal pathogenic *E. coli* (ExPEC), including avian pathogenic *E. coli* (APEC), pose a considerable threat to both human and animal health due to potential economic losses stemming from illness. APEC strain chi7122 (O78:K80:H9), originally isolated from the liver of a diseased turkey, has been used for many years as a model strain to study the molecular mechanisms of ExPEC pathogenicity and zoonotic risk.

Multiple large plasmids are a defining feature of APEC. In order to fully understand their functions in *E. coli*, we fully sequenced and characterized three large plasmids of APEC chi7122, including pAPEC-1 (IncFIB/FIIA-FIC, 103 kb), pAPEC-2 (IncFII, 82.7 kb), and pAPEC-3 (IncI2, 56.7 kb). The sequence analysis of the three plasmids has shown that although pAPEC-1 encodes for virulence genes common among APECs, including four iron acquisition systems (*iutA iucABCD, sitABCD, iroBCDN*), and temperature-sensitive hemagglutinin (*tsh*), a colicin V operon, increasing serum sensitivity (*iss*), *ompT*, *hlyF*, and *etsABC*, and pAPEC-2 for an ABC iron transport system (*eitABCD*), new features have been identified in the sequence of these plasmids, such as an unusual L-idonate pathway in pAPEC-2 with no similarity at the DNA level with other *E. coli* sequences and a type IV fimbriae encoded in pAPEC-3. Evaluation of strains with the three plasmids either individually or in combinations in vitro provides new insights into their roles in the interaction of bacteria with host cells, biofilm formation, bile and acid tolerance and show for the first time that both the nature and combinations of plasmids have an effect on these phenomena.

Characterization of APEC Isolates from Broilers in Latin America

Taylor M. Barbosa, Elizabeth Turpin and Lisa K. Nolan
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Avian Pathogenic E. coli (APEC) is the causative agent of avian colibacilosis. Genes encoding certain virulence factors can be used to differentiate APEC from commensal E. coli . Multiplex PCR tests that target these differentiating genes have been established and used to study E. coli from North America poultry Here, we use these tests to characterize APEC from several countries in Latin America in an effort to determine if APEC from North and Latin America share similar virulence attributes.

Pathogenicity of Mycoplasma gallisepticum strains using ELD50 in embryonated chicken eggs

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Mycoplasma gallisepticum (MG) is a respiratory disease affecting poultry. The molecular determinants of virulence for MG are largely unknown and pathogenicity studies in chickens are time consuming, cost prohibitive, and facility and labor dependent. Our objective in this study was to evaluate the ELD_{50} method to screen selected MG strains for pathogenicity. This method has been used in the past with variable results. We compared the ELD_{50} of various "ts-11-like" field isolates of MG with ts-11 (an apathogenic vaccine strain) and R strain (a highly pathogenic challenge strain).

11137

Evaluation of Three DNA Extraction Methods for the Detection of Mycoplasma Spp. with an MG/MS Multiplex Realtime PCR Method

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Nucleic acid extraction assay limitations are an important concern in diagnostics as they are paramount to the success of pathogen identification. Extraction efficiencies vary among methods thus giving varying DNA yields and quality and careful consideration needs to be made when choosing the appropriate extraction assay. Our goals were to: 1) compare three DNA extraction methods for Mycoplasma gallisepticum (MG) and M. synoviae (MS) and determine their suitability for use with the VetMAX multiplex MG/MS realtime PCR method; and 2) evaluate the effect of DNA storage time and temperature on MG and MS detection. Our samples included actively growing pure cultures of MG and MS reference strains as well as tracheal swabs from infected chickens. The DNA quantity varied by method and decreases in quality and quantity of DNA were observed over time at both 4 and -80oC with the most significant decreases occurring at 4oC. Storage time and temperature also had an effect on Ct values. Overall, the results reveal important differences among the DNA extraction methods that are relevant for the success of downstream molecular analysis.

11138

Experiences in the Use of a Live MG Vaccine in Northeast Georgia

Len Chappell
Georgia Poultry Laboratory Network

To determine the incidence of vertical transmission of live TS-11 vaccine (Merial Select Inc.) to progeny broiler flocks, five young, TS-11 vaccinated, breeder flocks, from a major poultry company in Georgia were bled and swabbed every eight weeks for the life of the flocks. These five flocks collectively represent a typical TS-11 vaccinated flock in Georgia. Thirty swabs and blood samples from more than eighty broiler flocks, from TS-11 vaccinated breeder flocks, were collected at the processing plant. Many of these broiler flocks were from the five targeted breeder flocks. With the use of PCR: MG and sequencing, the overall rate of vertical transmission (%) of TS-11 vaccine from breeder to broiler populations was determined.

Use of MG/MS Serology and PCR to Determine Flock Status

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Georgia Poultry Laboratory Network

Each serum sample submitted to GPLN for MG and MS serology is run on the MGMS ELISA. All ELISA positive samples are then run on MG and MS HI s. If samples are positive on HI s, the customer is asked to submit 30 additional sera and 30 swabs from the flock for further testing. The sera is then tested again by the protocol previously described. The swabs are tested by real time PCR for the presence of MG or MS antigen. This paper will evaluate and compare the ELISA, HI, and PCR data obtained during a 12 month period and how this data is used to determine the positive status of MG or MS flocks.

11140

Expanded Sequencing of *Mycoplasma synoviae vlhA* Gene as a Complementary Genotyping Tool

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Intraspecific differentiation of *Mycoplasma synoviae* (MS) is essential for epizootiological investigations. So far only one genomic traget, the upstream reagion of the *vlhA* gene, was idenitified for MS genotyping by sequencing. This study describes the successful amplification of an expanded segment (over 1200 bps) of the *vlhA* gene in DNA extracts from MS laboratory strains and positive clinical cases. Sequence analysis of the expanded *vlhA* segment allowed for better differentiation of clinical MS cases. This new assay can serve as a complimentary genotyping tool in MS outbreak investigations and epizootiological studies.

11141

A Survey of Recent Mycoplasma synoviae vlhA Sequence Types in the Southeastern United States

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Mycoplasma synoviae (MS) is a poultry pathogen that has a surface lipoprotein (vlhA), not only responsible for cytadhesin, allowing infection, but is also useful for strain differentiation. In this survey, vlhA sequence data from 2005 to present were collected from 147 independent avian clinical cases from 7 Southeastern U.S. States. A total of 27 different vlhA sequence types were identified. This survey will identify sequence types associated with broilers, layers, turkeys, or backyard birds. We will examine the incidence of sequence types and show their prevalence in geographic locations.

11143

Histopathology, Immunohistochemistry and Cytokine Responses in Gangrene Dermatitis-Affected Chickens

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The present study was undertaken to investigate pathological and immunological changes, and molecular diagnosis associated with gangrenous dermatitis (GD) outbreaks in broiler chickens. Ten birds with clinical GD symptoms and 5 control birds which appear clinically-healthy were selected for sample collection to measure nitric oxide (NO), acute phase protein (AGP), tissues for histology and immunohistochemistry and cytokine transcripts. GD birds demonstrated typical clinical symptoms, gross lesions at necropsy and histopathological findings included hemorrhagic lesions, degeneration, and necrosis of parenchymatous cells, especially of skin, muscle, and intestine. Immunofluorescence staining revealed Clostridium-like bacilli in the skin and intestine. C. perfringens (CP) and C. septicum (CS) genomic sequences were identified by PCR in samples from skin, muscle, and intestine. Serological analysis demonstrated that both diseased and healthy birds had high antibody titers against CP, CS, Eimeria, chick anemia virus, and infectious bursal disease virus. Mitogen (ConA, LPS)-driven splenic lymphocyte proliferation was significantly depressed in GD birds compared to control birds and GD chickens produced higher levels of serum NO and Â±-1-AGP. Flow cytometric analysis of lymphocyte subpopulations showed K55-, K1-, CD8-, MHC II- positive cells in the skin, and K55-, K1-, CD8-, TCR1-, TCR2-, Bu1-, MHC II- positive cells in the intestine of GD birds. The expression levels of mRNAs encoding pro-inflammatory and chemokines were increased in GD birds. These results provide histological, immunological and molecular changes associated with GD infection in broiler birds, especially document changes associated with innate immune response to Clostridium pathogens in GD in broiler chickens.

11144

Toll-Like Receptors and Cytokines Profile of Chicken Challenged with Clostridium perfringens and Fed Organic Diets Supplemented with MOS

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A ban in antibiotic growth promoters (AGP) in poultry diets has resulted in the re-emergence of necrotic enteritis (NE) caused by Clostridium perfringens, a gram positive bacteria ubiquitous within the environment and the avian intestine. Mannan-oligosaccharides (MOS), an alternative to AGP, can decrease levels of C. perfringens in the gut and mortality associated with NE. However there is scant information supporting the possible immunological responses involved in its potential activity. The present study tested the effect of different concentrations of MOS on ileum and cecal tonsil gene expression of toll-like receptors (TLR) 2 and 4, and cytokines IFN-gamma, interleukin 6 (IL-6), IL-10 and IL-12 in C. perfringens challenged broilers. Four groups of 25 broilers, Ross x Ross, were randomly assigned to six dietary treatments; challenge (positive control) and unchallenged (negative control), and a diet containing 0.2, 0.4, 0.6, and 0.8% MOS, and were fed ad libitum for 21 days. Samples were collected at day 14 (pre-challenge) and 21 (7 days post-challenge). Data was analyzed with the mixed procedure of SAS and means subjected to analysis of variance. Pre-challenge there was no significant difference in TLRs and cytokines profile between groups. A significant up regulation in TLR2 ($p<0.05$) in both Ileum and cecal tonsils was observed in the 0.8% MOS group post-challenge. No significant difference in gut location associated TLR4 gene expression and cytokines profile was observed between groups. However, high levels of IFN-gamma and IL-10 were observed in cecal tonsils post-challenge in all groups indicating a strong inflammatory process involving the innate immunity.

11145

Characterization of Clostridium septicum Isolates from Cellulitis Cases in Turkeys

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Clostridium septicum is an important pathogen of animals and humans. It has been isolated from cases of cellulitis in turkeys. Our earlier studies have shown Clostridium septicum toxoid to be protective against cellulitis in turkeys. It is important that we examine the immune reactive components of C. septicum that play a role in immunity. The objective of our study was to characterize the C. septicum isolates and also to identify their secretory components that may elicit protective immune response in turkeys. In brief, C. septicum isolates that appear to be potent in causing cellulitis and those which did not produce cellulitis were included in this study. They were grown in sporulation media and allowed to express toxins. The culture supernatant from these isolates was subjected to SDS-PAGE analysis and two-dimensional gel electrophoresis to separate the proteins. A western blot was performed using convalescent sera from the birds exposed and non-exposed to C. septicum toxins. The reactive toxin components were identified by MALDI-TOF mass spectrometry. Our results suggested involvement of different toxins of Clostridium septicum that may play a role in pathogenesis and protective immune response against cellulitis in turkeys.

Dexamethasone Model for Cellulitis in Turkeys

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Cellulitis / Clostridial dermatitis is an emerging disease in commercial turkeys in Minnesota and elsewhere. Most often, the bacterial agent isolated belong to Clostridium septicum. There is very little information as to the exact and to what extent Clostridium septicum play a role in Cellulitis in turkeys. Our objective in this study was to develop an oral/subcutaneous challenge model with C. septicum for Clostridial dermatitis in turkeys in a dexamethasone -immunosuppressed background. We immuno-suppressed 10-week old turkey poults with a chemical Dexamethasone intramuscularly. The birds treated with Dexamethasone and not-treated were then challenged with C. septicum orally and subcutaneously to examine the development of Clostridial dermatitis. The Dexamethasone treated birds were found to be more susceptible to C. septicum Challenge and developed cellulitis than the non dexamethasone treated birds. This could be used as a disease model for testing vaccines against cellulitis.

Effects of Yeast Extract and Vitamin D on Turkey Mortality and Cellulitis Incidence in a Transport Stress Model

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We evaluated yeast extract (YE) and vitamin D (VD) in turkeys treated with dexamethasone (Dex) at intervals designed to simulate transport stress during a 3 stage growout. YE but not VD decreased early mortality (P = 0.001) and mortality at wk 7 (P= 0.02) and wk 12 (P = 0.002) but not wk 16. Cellulitis lesions consistent with Clostridial Dermatitis (CD) were seen in 8/21 birds that died and were decreased by YE during weeks 13-16 (P = 0.04). These data suggest that transport stress may play a role in CD and that YE may help counter the effects of stress.

Use of a Repeatable Model Creating Significant Clostridium Dermatitis Mortality in Turkeys to Determine Management and Other Risk Factors that Affect the Severity of the Disease

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Clostridium Dermatitis or C. septicum caused gangrenous dermatitis causes severe disease and mortality in commercial turkey production in the USA and the disease has proven to be very difficult to reproduce in a research model. A model has been developed and repeated that causes 30-60% Clostridium Dermatitis mortality in tom and hen turkey flocks by exposing naÃ¯ve turkeys to C. septicum contaminated used litter similar to practices used by commercial turkey operations. The repeatable high incidence of mortality has provided an opportunity to compare environment conditions such as litter moisture and room temperatures etc. to evaluate the environment conditions and other management practices impact on the incidence of Clostridium Dermatitis caused mortality in a controlled setting. Results of environment and management conditions impact on mortality will be presented. The application of this model provides valuable information in possible mechanisms to help control this disease and provides a method to evaluate a wide range of potential preventative products and/or management practices.

Kinetics of Anti-Cryptosporidia Antibody Response, Oocyst Shedding and Bursa/body Weight Ratios in SPF White Leghorn Chickens Infected With Cryptosporidium Baileyi Oocysts at Different Ages

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The aim of this study was to investigate and compare the response of SPF birds to an infection with Cryptosporidium baileyi (C. baileyi) oocysts when inoculated at the age of one, 4, 11, 29 or 60 days. For each inoculation age, one group of birds was inoculated with $5x10^5$ C. baileyi oocysts and compared to an uninoculated control group. Sera were sampled weekly and examined individually for the presence of different isotypes of immunoglobulins (IgG, IgM, IgA and total immunoglobulins) against C. baileyi by ELISA. Individual samples of feces were monitored every other day for oocyst shedding by the MSF method as described previously (Abbassi et.al. 2000). Five birds per group were sacrificed each week and the bursa/body weight ratios were measured. The remaining birds in the groups infected at one and 11 days of ages were challenged with 10^6 oocysts at day 57 and day 60 respectively. The same criteria were studied in these challenged groups and compared to the corresponding uninoculated control groups. For the antibody response, a stronger more lasting response was detected earlier in the older birds for all immunoglobulin isotypes. For the oocyst shedding, the prepatent period was longer and the patent period was shorter in the older birds. A clear atrophy of the bursa of Fabricius was detected in the group inoculated at one day of age showing significantly lower bursa/body weight ratios and confirmed by histology. A late infection has less to no effect on the bursa of Fabricius.

11150

Effects of novel nanoparticle adjuvant Montanide™ IMS 1313 N VG on Mucosal Vaccination of Poultry Against Eimeria Acervulina

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The present study was conducted to compare the immune enhancing property of nanoparticle adjuvant, IMS 1313 N VG PR (IMS1313) with the Montanide" ISA 71 VG (ISA 71) on recombinant profilin vaccination against avian coccidiosis. Day-old male broiler chickens were immunized subcutaneously or by non-parenteral routes (oral, nasal and ocular) with profilin plus complete Freund s adjuvant (CFA), Montanide ISA71 VG, or IMS 1313. Broiler chickens were orally challenged with E. acervulina oocysts and their growth performance, fecal oocyst shedding, and acquired immunity to profiling were evaluated. Immunization with profilin plus ISA71 or IMS1313 induced significant enhancement in secretary IgA and IgY responses compared with non-vaccinated or profilin/CFA-vaccinated groups. Profilin plus Montanide ISA 71 injection induced significant improvement in body weight gain and reduced fecal oocyst shedding with enhanced presence of intraepithelial lymphocytes (IELs) expressing CD4, CD8, TCR1 and TCR2 in the duodenum compared with the control groups. These results demonstrate for the first time immune enhancing properties of novel adjuvants such as Montanide" ISA 71 VG and IMS 1313 in recombinant subunit vaccination against coccidiosis.

11151

Essential Role of Avian Interleukin (IL)-22 as Iimmune Mediator During Inflammatory Response

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A member of the interleukin (IL)-10 family, IL-22 is an important effector of activated Th1 and Th17 as well as natural killer cells during inflammatory responses. Interestingly, recent studies show little involvement of mammalian IL-22 in communication between immune cells. Rather, it mainly acts on epithelial cells, where it functions in antimicrobial defense, regeneration and protection against damage, as well as inducing production of acute phase reactants and chemokines. This study reports the cloning of recombinant avian IL-22 and characterization of its biological effects during stimulation of immune response. The full-length of avian IL-22 was amplified and cloned into either a prokaryotic (pET28a) or a eukaryotic (pcDNA3.1) expression vector. The bacterially expressed avian recombinant IL-22 (rIL-22) was purified and the endotoxin removed. Using endotoxin-free rIL-22, expression of pro-inflammatory cytokines and Th1/Th2 cytokines was measured using quantitative real-time PCR (qRT-PCR).

Glycoprotein J, I, and E specific ELISA for the detection of ILT

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Commercial indirect ELISA for the detection of ILTV antibodies in chicken sera are currently based on virus purified from chicken cell cultures which results in high background. After heterologous expression of recombinant glycoproteins I (gI), E (gE), and J (gJ) of ILTV in a baculovirus expression system the antigens were highly purified from supernatants of insect cell cultures. Engineering of the recombinant glycoproteins, establishment of ELISAs, and validation using field sera are presented. While the recombinant gE and gI yielded the highest amounts after expression, recombinant gJ proved to be the best antigen for high sensitivity and specificity in ELISA.

11153

Exploration of the Early Mechanisms Involved in the Iimmune Protection Against the Infectious Laryngotracheitis Virus Infection

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Infectious laryngotracheitis (ILT) is a highly contagious respiratory disease of chickens which causes severe production losses to the poultry industry worldwide.
The objective of this study was to evaluate the expression of key genes involved in the host immune response during the early hours after infection. Changes in mRNA expression levels was measured using real time RT-PCR in 4 week-old specific pathogen free chickens within treatment groups: i) vaccinated and challenged; ii) non-vaccinated and challenged; iii) vaccinated and non-challenge; and iv) non-vaccinated non-challenged. Comparison among groups showed significant differences in mRNA expression for IFN-Â³ and IL1-Â².

Whole Genome Sequencing of Infectious Laryngotracheitis

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Infectious laryngotracheitis (ILT) is a herpesvirus-induced acute respiratory disease of world-wide importance to the poultry industry. ILT was the first major avian viral disease for which a vaccine was developed in the 1930s. In its acute form, ILT is characterized by signs of respiratory distress in birds, accompanied by gasping and expectoration of bloody exudate. In addition, the mucous membranes of the trachea become swollen and hemorrhagic. The epizootic form of the disease spreads rapidly and can affect up to 90-100% of an infected flock, while mortality generally averages between 10 and 20%. The etiological agent for ILT is infectious laryngotracheitis virus (ILTV). ILTV (Gallid herpesvirus 1) is an alphaherpesvirus and a member of the Iltovirus genus. Despite its history, the first complete sequence of ILTV (in silico) was reported in 2006. ILTV molecular diagnostics have been hampered due to an inability to clearly differentiate field viruses from vaccine viruses. This is probably due to the fact that most live attenuated vaccine strains of ILTV were derived from field strains and the relatively stability of the ILTV genome. The goal of this experiment is to generate and compare the complete nucleotide sequences of several Delmarva field isolates of ILTV which have been collected over a 20 year period (1985-2005). We have now determined the complete nucleotide sequence of a chicken embryo origin vaccine strain of ILTV (CEO), a tissue culture origin vaccine strain (TCO) and an ILTV field isolate from 1985 (632). Analysis of these sequences will help to determine the relative stability and/or evolution of the ILTV genome in one geographic area, and when compared to the sequence of commonly used vaccine strains may lead to the development of better diagnostic tools. Our results currently suggest that these ILTV strains share >98% sequence identity with the reference ILTV sequence. However, the TCO vaccine strain and the 1985 field isolate share an ~3.0 kb deletion in the unique long region of the genome which is not found in the CEO vaccine. SNP analysis and sequencing of more contemporary ILTV field isolates is in progress. Currently we have generated sequence from a (ILT) CEO, TCO vaccine strains and a ILTV field isolate from 1985. These sequences along with the USDA challenge virus sequence gives us a unique opportunity in determining the frequency of genetic markers from different ILT sequence sources.

South Carolina Ag-Watch Program – An Awareness and Response Program for Food & Agriculture Producers, Processors and Responders

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The SC Ag-Watch program was developed for South Carolina's animal, plant and crop producers, food processors and agricultural responders who must prepare and protect themselves against threats against their livelihoods such as disease/pest transmission, food contamination, agroterrorism, and natural disasters. The goal of this program is to train them to be sentinels in early detection and notification which will serve to harden potential food and agriculture targets and will strengthen response by regionalizing an event and implementing business continuity planning. Program components are training sessions (for animal, plant/crop producer and food processor audiences); development of a reference manual; biosecurity/site security audits; and exercises. Training topics include an overview of foreign animal diseases, exotic plant diseases and pests, and food defense as well as biosecurity/site security procedures and proper notification protocols. The SC Ag-Watch Manual, distributed to training attendees, was designed to be a producer/processor-friendly resource guide for SC stakeholders, with biosecurity sections written in a species or commodity oriented format. Voluntary biosecurity/site security audits can be requested by producers and processors who have completed the training and who wish to be designated as an “SC Ag-Watch Site.” Exercises conducted thus far utilized foreign animal disease and exotic plant diseases/pest scenarios and involved private industry and multiple public health and food and agriculture agencies.

11156

Effects of Egg Remover on Bone Development at Hatch, and Male Broiler Live Performance and Leg Health at Market Age under Commercial Conditions

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Bone development and leg problems can be affected by temperatures during the last days of incubation. One experiment was conducted under commercial conditions to evaluate the effects of removing unfertile eggs to reduce egg mass and improve ventilation in hatchers. In a commercial hatchery eggs from 35 to 45 wk-old breeder flocks were split from 8 Chick-Master setters to hatchers at 19 days. During transfer half of the trays of each setter and breeder flock pass through the egg remover to pick clear eggs and the other half were non picked . At hatch, 32 chicks per treatment were selected. Body weights (BW) with and without yolk, and bone traits were collected immediately after hatch. 20,100 male broilers from each treatment were placed in tunnel houses. Individual BW were collected at 7 and 56 days of age. Leg health issues and gait scores were evaluated at 56 days of age. Results indicated that at hatch, chicks coming out from picked hatchers were heavier than chicks from non picked treatment. Chickens from non pick treatment had more femur and tibia bone as proportion of their BW without yolk at hatch, but there bones were more asymmetric. At 7 days, the non picked treatment had heavier chickens (153 vs 150 g), but at 56 days of age broilers coming from picked hatchers were significantly heavier (3,857 vs 3,772 g). Flock uniformity and livability were also improved by pick treatment. No significant effects of treatments were observed on gait scores or leg health issues.

VIRULENCE EVOLUTION OF MYCOPLASMAL CONJUNCTIVITIS IN HOUSE FINCHES

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We are working to understand the evolution of *Mycoplasma gallisepticum* virulence in the ongoing epidemic of House Finch (*Carpodacus mexicanus*) conjunctivitis. These investigations were motivated by the observation that prevalence rates were far lower in western than eastern North America, and that disease prevalence had also declined in eastern North America over time. Our goal was to understand whether these spatial and temporal differences in disease prevalence were the result of host and/or pathogen evolution. We compared the responses of two populations of House Finches (east and west of the Great Plains) to experimental infections with one of two isolates of *M. gallisepticum*: the original isolate made in 1994 in Virginia, and a 2006 isolate from northern California. We found that differences in virulence were entirely caused by differences between the two isolates of *M. gallisepticum*, indicating that pathogen evolution was the predominant reason for virulence variation in House Finches. This opened the question as to whether the pathogen has evolved through space (from emergence in the mid-Atlantic to the Pacific coast), or time (decline in virulence in both eastern and western North America). To answer this question we compared the virulence of two eastern North American isolates of *M. gallisepticum*: the original 1994 isolate from Virginia and a 2006 isolate from North Carolina. We found increased virulence with the 2006 isolate compared to the 1994 isolate. In eastern North America, *M. gallisepticum* has apparently evolved towards increased virulence since its emergence as a pathogen of House Finches.

11158

Phylogenetic Analysis of Mycoplasma Synoviae Isolated From the Chickens With History of Clinical Signs

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Eunok Jeon

Mycoplasma synoviae (MS) is a major pathogen of poultry industry causing respiratory distress, synovitis and eggshell abnormalities. However, it was difficult to reproduce characteristic clinical signs of MS in the laboratory because of variable virulence of MS and the involvement of other factors. Therefore we tried to isolate the MS from the field cases with history of clinical signs to compare the virulence of isolates. We isolated MS from the samples of choanal cleft and tracheal swab and conducted phylogenetic analysis about their vlhA gene which encode a surface protein, a haemagglutinin VlhA and is believed to play a major role in pathogenesis of the disease. Total 16 MS were isolated and subtyped using vlhA gene sequence.

Among 16 isolates, 1 isolate is type C, 2 isolates is type D, and 4 isolates is type E. The rest 9 isolates did not belong to any previous classification because the isolates contain 35 amino acids in the PRR region. We classified these isolates as new type G. In the further study, we will try to reproduce the characteristic clinical signs of MS and evaluate the relationship between vlhA gene and virulence of MS in the SPF chickens.

11159

Sequencing of South African *Mycoplasma gallisepticum* Isolates Reveals Novel Genotypes

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Mycoplasma gallisepticum (MG) is primarily a respiratory pathogen, which causes a disease of considerable economic significance to the poultry industry worldwide. The organism exhibits extensive genetic diversity, and there is a wide variation in pathogenicity between strains. In South Africa, MG has been associated with severe respiratory disease, sometimes in the absence of other respiratory pathogens. We conducted polymerase chain reaction (PCR) and sequencing of genome targets shown to be useful in strain differentiation of multiple MG isolates collected from commercial poultry in a range of different locations within South Africa. This has revealed novel MG genotypes, whose sequences are distinct from the MG sequences from other countries. These results allow a better understanding of the diversity and epidemiology of MG isolates within Southern Africa, and their relatedness to isolates from other countries, with possible implications for the selection of diagnostic tests and for the control of this disease.

Further Assessment of the VG/GA Newcastle Disease Virus Strain for In Ovo Vaccination in Commercial Broilers

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Previous work indicates the VG/GA strain appropriateness for in ovo application. Further assessment included: VG/GA (AVINEW®) in ovo or at hatch with and without day-one killed vaccination. LaSota strain was applied (seven and 17 days) to all but the unvaccinated control group. A stringent genotype VII NDV lethal challenge was applied (28 days). When compared with the controls, protection was significantly higher ($P < 0.05$) in all vaccinated groups, regardless of the initial vaccination route. A 90,5% protection was achieved in the groups including the killed vaccine. No significant differences between in protection when using *in ovo* or spray initial vaccination corroborates previous observations on the suitability of the VG/GA for the *in ovo* route.

Effect of Newcastle Disease Virus (La Sota strain) Infection on Chicken Respiratory Macrophage

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Chungbuk National University

The alveolar macrophage is one of the key cells for defense mechanism of mammalian species against infection of various organisms. However, the respiratory macrophage of avian species has not been studied intensively yet even respiratory system is the major route of infection of various organisms including Newcastle disease (ND). Previously, the non-specific immune stimulation potential of ND virus was well described in several mammalian species. But it is not clear whether this potential is also validatable in avian species. Therefore, we conducted this study to evaluate the defense mechanism of avian respiratory macrophage (ARM) against ND viral infection. We inoculated La Sota strain, one of the ND vaccine virus, in nasal cavity of 4 weeks old specific pathogen free (SPF) chickens, and migration, phagocytic and bactericidal activity of ARM were measured. . At 1,3,5,7 days post inoculation in the SPF chickens, we lavaged lung using sterile PBS through feeding tubes and respiratory macrophages were collected from lung lavage fluid by centrifugation and RBC lysis. Slight increase in bactericidal activity was observed in the ND virus inoculated group at 1, 3 and 5 dpi compared to those of non-inoculated group. However, only the bactericidal activity at 1 dpi was statistically significant ($P < 0.05$). This result suggests that ND virus inoculation can induce non-specific immune stimulation effect such as bactericidal activity on avian respiratory macrophage. However, we need further studies for the mechanism of stimulators such as nitrogen oxide and H_2O_2 to precise evaluate the bactericidal activity of ARM which found in this study.

11163

Characterization of Avian Paramyxoviruses Isolated From Migratory Waterfowl in Chickens, Turkeys, and Ducks

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Avian paramyxoviruses (APMV) are common viruses of domesticated and feral birds. Ten distinct serotypes, including APMV-1 (Newcastle disease virus) have been recognized in various avian species found worldwide. Monitoring migratory bird populations is useful in identifying viruses that may pose disease threats to commercial poultry. As part of the Highly Pathogenic Avian Influenza Early Detection Data System (HEDDS), results from over 8,000 wild birds sampled on the Delmarva Peninsula have been compiled (HEDDS, 2010). In addition to isolating numerous avian influenza viruses, APMV isolates were recovered from migratory waterfowl. The poster will detail the characterization of APMVs isolated from wild bird surveillance efforts in broiler chickens, commercial turkeys and domestic mallard ducks.

11164

Avian Paramyxovirus Serotype 1 Strains of Low Virulence With Unusual Fusion Protein Cleavage Sites Isolated From Poultry Species

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Avian paramyxo-serotype-1 viruses (APMV1) with fusion cleavage sites containing two basic amino acids and a phenylalanine (F) at position 117 have been isolated from poultry species in two states from 2007-2009. The intracerebral pathogenicity indices for these viruses are of low virulence at 0.00 and 0.04. The isolates have fusion protein sequences that are 97% similar to wild bird NDV strains on nucleotide and amino acid levels. The mean death time for chicken embryos ranged from 109-1271hrs and for turkey embryos 67-127hrs. Reported here are phylogenetic analyses for the nucleocapsid, phosphoprotein, matrix, fusion, hemagglutinin-neuraminidase, and polymerase genes.

Analysis of Transcriptional Cytokine Responses of Chickens Infected With different Newcastle Disease Virus Isolates Using Formalin-Fixed Paraffin-Embedded Samples

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Innate responses are considered important during the earliest phases of microbial invasion because they can limit the spread of the pathogens. Measurement of cytokine responses during infection in vivo can help to elucidate the mechanisms of virus pathogenesis. The transcriptional response of several cytokines in the spleen of chicken naturally infected by NDV velogenic viscerotropic viruses was compared to the responses of atypical velogenic, velogenic neurotropic, and mesogenic strains during the first five days after infection. The RNA expression for IFN-gamma and IL-6 was enhanced at day two in the highly virulent velogenic viscerotropic viruses (California and rZJ1 strains) and corresponded with the presence of the virus in tissues. However, in one atypical velogenic viscerotropic virus (Australia strain), two velogenic neurotropic viruses (Turkey ND and Texas GB) and, a mesogenic virus (Anhinga strain) the cytokine responses to infection were delayed or reduced. Increased levels of IFN-beta RNA expression were only detected in one velogenic viscerotropic virus infected chicken (California strain) and one mesogenic strain (Anhinga) early in infection. The RNA expression levels of IL-2 did not increase upon infection with any of the viruses. A pronounced increase of RNA expression levels of IL-6 and IFN-gamma was detected simultaneously with infiltration of macrophages and/or lymphoid necrosis in the histopathological analysis of the spleen and cecal tonsils. The differences in the RNA expression levels may help explain possible underlying mechanisms of clinical disease and/or immune responses in birds infected with different strains of APMV-1 that cause distinct pathologic changes.

11166

Exchanging the Fusion and Hemagglutinin-neuraminidase Genes of Newcastle Disease Virus: Effects on Virulence

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Newcastle disease (ND) is a severe disease of poultry and other avian species characterized by high morbidity and mortality. The etiologic agents are the virulent strains of Newcastle disease virus (NDV), which are part of the Mononegavirales class, Paramyxoviride family, Avulavirus genus. The number of virulent strains of NDV reported worldwide has increased in the last decades and these have been classified into ten different genotypes. The simultaneous circulation of multiple genotypes in the field and the widespread use of live virus vaccines raises the possibility of creating new recombinant viruses displaying unexpected virulence by natural recombination. We have used recombinant DNA technology to study the compatibility of genetic exchanges among viruses of different genotypes and to understand how those replacements affect virulence. We have exchanged the two surface genes of viruses of three different genotypes into a common NDV backbone containing the nucleocapsid, phosphoprotein, matrix and polymerase. The Fusion and the Hemaglutinin-Neuraminidase genes from viruses of genotype V, VI and VII have been replaced into the backbone of the Genotype II LaSota vaccine virus and the effect of those replacements on virulence and host responses analyzed. Mean time to death in eggs, intra-cerebral pathogenicity index in one day old chickens, overall pathogenicity, and transcriptional host responses in four week old chickens are described.

Comparison of Microscopic Methods for Bursa Histopathology Evaluation

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The microscopic pathology of 600 bursa samples from four broiler farms was evaluated using three methods [simple generalized subjective histopathology scoring, additive detailed histological scoring and quantitative measurement of optical density]. In the first method, all bursas were given a subjective severity score for overall histopathology using a range of 0 to 5 plus [0 = Absent; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked, and 5 = Severe]. The detailed cumulative subjective histopathology scoring method involved first assigning individual scores to multiple subcategories of microscopic pathology and than generating a generalized histopathology score by summation of the component scores. The following parameters were scored: lymphoid depletion; surface scalloping ; heterophilic inflammatory infiltration, interstitial inflammatory cell infiltration, edema, fibrosis, and cystic changes. The third method employed was to measure the optical density of gray scale photomicrographs of the individual bursas. A total of six bursas per house were submitted for evaluation in the studies. While all bursas were used for simple generalized histopathology scoring, only one of the six bursa sections for house groups were evaluated using the other two methods. When obvious differences were present between bursas within individual house groups, the most severely involved bursa was used for evaluations. However, the findings for individual birds within house groups were generally similar. The results for comparison of the three methods will be presented.

Testicular and Epididymal Lesions in Broiler Breeders

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Maintaining fertility late in the production cycle of broiler breeders can be difficult. Adding new males ("spiking") to the flock is often done to improve declining fertility suggesting males are primarily responsible for the problem. In this study testes and epididymides from 3 broiler breeder flocks, 57 – 68 weeks of age, raised at the Chicken Educational Unit at North Carolina State University for research purposes were examined for microscopic lesions at the conclusion of their production cycles. Flocks had normal fertility; individual male fertility was not determined. Two breeds (Cobb, Ross) and two management systems (Cages, Floor Pens) were represented in the study. Caged males were individually housed and unable to mate naturally in contrast to males in floor pens that were mixed with females. Lesions in the testes included degeneration of the seminiferous epithelium, sperm retention, aggregated sperm masses, focal lymphocytic orchitis, and sperm granulomas. Lesions in the epididymides were more common and included ectopic seminiferous tubules or epithelium, aggregated sperm masses, sperm stasis, intraluminal foamy macrophages, calculi, cysts, sperm granulomas, and focal or interstitial lymphocytic epididymitis. No tumors were found in the testes, but small Sertoli cell tumors rarely occurred in the epididymis. Lesions in the testis and epididymis did not correlate with each other. No differences were found between the two breeds. Males kept in cages had significantly more epididymal lesions compared to those in floor pens suggesting normal breeding is necessary for maintenance of reproductive health. Discovery of lesions in the testes and epididymides in a relatively high percentage of broiler breeders was unexpected. How they may relate to the fertility decline that characterizes aging male broiler breeders is unknown.

An Efficient Method of Blood Collection From Poultry Presented for Necropsy

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Poultry veterinarians, diagnosticians and researchers are often presented live poultry for euthanasia and necropsy. Procedures performed commonly include the collection of blood and serum for serology. Methods that have been used to collect blood from these birds include a cardiac stick and lancing or venipuncture of the wing vein. This report demonstrates a rapid and efficient method of collecting blood samples from the femoral artery and vein immediately following euthanasia.

11170

Evaluation of Lymphoid Tissues From Broilers With Runting and Stunting Syndrome

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Runting and stunting syndrome has been prevalent in broiler companies throughout the U.S. since early 2004. Most notably, RSS causes severe weight suppression during the first few weeks of age, lack of flock uniformity, diarrhea, distention of the intestines, and a significant increase in feed conversion. Cystic enteropathy is a hallmark lesion observed by histopathological examination of small intestine samples from affected flocks. Although descriptions of RSS date back to the 1970s, the etiologic agent(s) has yet to be identified. While unfounded, evidence suggests this is a multifactorial disease. The disease is reliably reproduced using filtered intestinal homogenates from RSS affected broilers implying a viral etiology. Although several novel viruses have been isolated, the clinical disease has not been reproduced by experimental infection. Much of the focus of this disease has been focused on identifying the etiologic agent/agents. In this study, we are focusing on the immunosuppressive consequences of RSS in affected birds. Using a previously described challenge model, we collected the Harderian gland, bursa, thymus and duodenum from RSS challenged and unchallenged birds on days 1 through 5. The tissues will be evaluated histologically and by in situ hybridization using chicken astrovirus, avian nephritis virus 1 and 2, and parvovirus riboprobes.

11171

Isolation, Purification and Full Genome Sequence of a Chicken Astrovirus Isolated From Broiler Chickens With Runting and Stunting Syndrome

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Chicken Astrovirus strain 4175 (CAstV-4175) was isolated, purified and propagated from broiler chickens affected with Runting and Stunting Syndrome (RSS). The full length sequence of CAstV 4175 displays a genomic organization characteristic of astroviruses, which contain three open reading frames (ORFs). ORF1a and ORF1b encode non-structural polyproteins 1a and 1b. ORF 2 encodes a viral structural polyprotein that is cleaved by an intracellular protease and forms mature capsid polyproteins. The genetic sequences of CAstV-4175 exhibit high similarity to sequences of turkey and duck astroviruses. Infection of young chickens with CAstV-4175 induced intestinal villus shortening and clubbing, and enterocyte vacuolation. Enteric infection with CAstV-4175 was localized to the apical and mid-villus enterocytes.

11172

Experience of using inactivated Salmonella Vaccines for Chickens, Salenvac and Salenvac T, in Europe

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Intervet Schering Plough Animal Health

The introduction of inactivated Salmonella vaccines, Salenvac and SalenvacT, produced under iron-restricted conditions and containing an aluminium hydroxide adjuvant, in the United Kingdom from 1997 and subsequently throughout other countries in Europe, along with additional control measures, has led to a significant reduction in the incidences of Salmonella Enteritidis and Typhimurium. Salenvac T is now currently available in around 40 countries worldwide.

The poster will describe studies demonstrating the efficacy of the vaccines against challenge, reduction in egg contamination rates and the improved performance of birds vaccinated with these vaccines compared to oil adjuvated vaccines.

11173

Development of an Enteric Virus Panel Test for Detection of Turkey Enteric Viruses

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A panel of two multiplex reverse transcription-polymerase chain reaction (RT-PCR) assays was developed for rapid and simultaneous detection of six major enteric viruses of turkey: turkey coronaviruses (TCoV), turkey astroviruses (TAstV; TAstV1 and TAstV2), avian enteroviruses (AEntV), turkey toroviruses (TtoV), avian reoviruses (AReV) and avian rotaviruses (ARoV; groups A, D, F and G). The first multiplex RT-PCR assay detected TCoV, TAstV, AEntV, and TtoV whereas the second assay differentially identified TAst1, TAst2, ARoV (Gps A, D, F and G) and AReV. The assay was optimized for use with intestinal contents/feces from turkeys. Each test was shown to be specific for the intended target and did not amplify other common RNA or DNA avian viruses. The detection limit was determined to be 8 ng of RNA used as starting template. This multiplex RT-PCR panel test proved to be a cost-effective tool for rapid simultaneous detection of turkey enteric viruses.

Protection Against Turkey Coronaviral Enteritis by DNA Vaccination

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The present study was undertaken to determine the immune response and protective efficacy of DNA vaccines against turkey coronavirus (TCoV) infection in turkeys based on TCoV nucleocapsid (N) and spike (S) genes. One-day-old turkeys were inoculated with one dose of 750 ug of TCoV N gene-based DNA first, followed by inoculating with 750 ug of TCoV S1 gene-based DNA two times at weekly interval. The turkeys that received one dose of N gene-based DNA and two doses of S1 gene-based DNA had significantly increased stimulation index for TCoV N protein-specific lymphocyte proliferation at 14 or 21 days after the first inoculation ($P<0.05$). ELISA titers to TCoV N protein or TCoV S1 fragment were low. The turkeys receiving one dose of N gene-based DNA and two doses of S1 gene-based DNA showed significant reduction or absence of TCoV antigens in the enterocytes by immunofluorescent antibody assay ($P<0.05$). The results indicated that DNA-mediated vaccination with TCoV N protein expressing DNA and TCoV S1 protein expressing DNA can elicit specific immune response to TCoV and reduce or inhibit TCoV infectivity in turkeys.

11175

Sequential Pathogenesis of Chicken Proventricular Necrosis Virus (R11/3 Virus) in Transmissible Viral Proventriculitis-affected Chickens

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An immunoperoxidase (IP) procedure was developed for detection of chicken proventricular necrosis virus (CPNV) using antisera generated against a recombinant, E. coli-expressed CPNV protein. Microscopic lesions characteristic of transmissible viral proventriculitis were detected in proventriculi on days 5 35 postexposure (PE) in CPNV-infected chickens; CPNV was detected by IP on days 1-14 PE in proventriculi. Early in infection (days 1-3 PE), viral antigens were detected primarily in mucosal epithelium of proventriculi; later (days 5 - 14 PE), antigens were detected primarily in glandular and ductal epithelium. No lesions or viral antigens were detected in tissues other than proventriculus.

11176

Preliminary Survey Of Hemoagglutinating Virus In Spotted Tinamou From Buenos Aires Province, Argentina

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A survey of spotted tinamou also call spotted nothura (Nothura maculosa) tissues and swabs harvested in the Province of Buenos Aires and inoculated for isolation of hemoagglutinanting agents will be reported. Since October 2008 samples such as cloacal and tracheal swabs plus a pool of organs have been harvested from freshly dead road kill birds or injured. All samples pooled according to date and area were inoculated in embryonated eggs via allantoic cavity. Up to now, no positive for hemoagglutination have been detected. While the material for inoculation have been obtained from dead or injured, but healthy free living tinamou, the first isolation of an avian influenza virus from the Tinamiformes order worldwide reported in Virology 396 : 76 84 (2010) was done from tissues belonging to clinically affected birds.

Propagation and Characterization of Turkey Reoviruses Isolated in Germany, 2004-2008

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From 2004 to 2008, suspected avian reoviruses were isolated from turkey flocks in ten counties in Germany. The age of birds at isolation ranged from 9 to 54 days. The suspected avian reoviruses elicited characteristic cytopathic effect (CPE) in chicken embryo kidney (CEK) cell culture. In 2009, CEK cell culture supernatants (n = 25) containing these turkey reovirus (TRV) isolates were shipped to the Southeast Poultry Research Laboratory (SEPRL, USDA/ARS) in Athens, GA for further characterization. After further propagation in CEK cells in BSL3-Ag facilities at SEPRL, the TRV isolates were inactivated using a TRIZOL RNA extraction procedure and nucleic acid was transferred to BSL2 laboratories for molecular diagnostics. RT-PCR primers designed to detect the TRV sigmaNS, sigmaC, and sigmaB genes were used to amplify cDNA from the German isolates. The German TRVs were, in general, phylogenetically distinct from United States chicken and turkey avian reovirus isolates. Based on the sequence obtained from the German TRV sigmaNS and sigmaB genes, two United States isolates one from North Carolina and one from Missouri grouped with the German TRVs. The United States TRV from North Carolina (NC/SEP-R44/03) has been previously characterized at the molecular level and in pathogenesis studies and has been implicated in immune dysfunction and enteric disease in poults.

11178

Identification and Molecular Characterization of Avian Reovirus in Commercial Broilers Using RT-PCR and Sequencing

Lanqing Li, Emily M. Handley, Michael R. Luther, Alicia Wise, Alana Fulmer, and Frederic J. Hoerr
Auburn University, Al

Different tissues from broiler showing clinical signs of enteric disease, the runting-stunting syndrome (RSS), and viral arthritis were collected for the detection of avian reovirus by RT-PCR. In this study, the S4 genes of 26 avian reoviruses were compared and analyzed by sequencing. Of 26 samples, 25 samples were collected from broilers, one from a commercial vaccine company. The amino acid sequencing results of S4 gene from 26 reoviruses shared 92.5 to 100% similarity with vaccine strains S1133 and1733. Only three field samples showed 100% similarity with vaccine strain 1733. Off 26 field samples, 22 samples had less than 95% (89-94.6%) similarity with vaccine strains in the S4 gene, and showed quite consistent difference at amino acid substitutions in S4 gene compared with vaccine strain S1133 and 1733, but more similar (94.6-100) to two field strains GA/790/05 and NC/837/05(Genbank reference).

11179

Comparison of fluorescent antibody and RT-PCR Testing for the Detection of Avian Reovirus in Clinical Diagnosis

Lanqing Li, Emily M. Handley, Michael R. Luther, Alicia Wise, Alana Fulmer, and Frederic J. Hoerr
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Fluorescent antibody (FA, from NVSL) and RT-PCR assays have been used to detect the avian reovirus from chicken tissue or cell culture as routine methods in TBS State diagnostic lab. Two tests have a high correlation when the virus is vaccine strain, but for field strains, the RT-PCR has much lower sensitivity than FA tests. This presentation will report the evaluation of the sensitivity and specificity of these two methods.

11180

Clinical, Pathological, and Virological Investigations of Multicentric Lymphomas Reported From Pheasants Flocks

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Several similar cases of multicentric lymphomas have been described in France among pheasants' flocks during last years. Affected birds were systematically older than 6 months, females being as affected as males. Clinical signs included mainly depression, anorexia and weight loss, followed by death within few days. A pathological and virological investigation was recently done. Main gross lesions included thickening of eyelids, neck and leg skin and less frequently, esophageal and crop wall. Necrotic membranes could be sometimes observed in the oropharynx. Histopathological examination of these organs revealed multifocal infiltration of lymphomatous cells admixed with few macrophages and heterophils. These infiltrations were sometimes associated with micro-hemorrhages and/or cutaneous necrosis. Therefore lesions referred to those usually seen in Marek's disease in chicken and virological investigations were processed. Virological analyses processed by PCR from eye-lid and neck skin excluded fowlpox virus infection; PCR detection of lymphoproliferative avian viruses is presented and discussed.

11181

Emergence of an Acute Chicken Fibrosarcoma Induced by Avian Leukosis Virus in China

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During recent years, myeloid leukemia and hemangioma in commercial layers and Chinese yellow meat-type chickens have commonly been reported in China. Tumor cases or lesions in the field were mainly found in sex matured chickens older than 16-18 weeks of age. Among about 120 Avian Leukosis virus (ALV) isolates, about 90% were identified as subgroup J (ALV-J) although some ALV isolates were also identified as subgroups A and B. However, field cases of acute fibrosarcoma were diagnosed in 30- to 40-day-old chicken flocks, which were hybrids of commercial layers with white meat-type males and raised for the meat purpose as yellow colour broiler. The fibrosarcomas with sizes of 3-5 cm in diameters were detected on the surface of necks in the most cases. The similar lesions were also reported in 2 commercial layer flocks with 10% mortality of myeloid leukemia and hemangioma at about 28 w of age. ALV-J was isolated from all fibrosarcoma cases, but co-infection of ALV-J and ALV-A was also recognized in some fibrosarcoma cases. When 1-day-old chickens of egg-type or meat-type were inoculated subcutaneously or intra abdomen with filtrates of tumor extracts from 40-day-old chickens or sex matured layers, similar tumors were detected in 10-12 days in almost all inoculated birds. These fibrosarcomas showed similar histology to those tumors from the field cases. In immunohistochemistry, almost all sections were positive with ALV-J specific monoclonal antibody JE9. It was also demonstrated that needles used in injection of infected 12-day-old chickens with gross fibrosarcomas could transmit the acutely transforming viruses to other birds and induce the same fibrosarcomas.

Infectious Bronchitis Virus in California 2003-2010: A Review

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Since 2003 more than 600 infectious bronchitis isolations from trachea/lung and cecal tonsil specimens, primarily from broilers have been made and the spike protein (S1) hypervariable region has been sequenced following RT-PCR amplification. A review of this amassed data will be presented.

Characterization of Infectious Bronchitis Virus Isolates from Backyard Flocks

Arun B. Kulkarni, Deborah A. Hilt, and M. W. Jackwood
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During recent investigations of GA08 IBV type viruses in commercial chickens in NE Georgia, four isolates were obtained from backyard poultry. In the present research, IBV genotypes from oropharyngeal swabs from apparently healthy backyard poultry from auction/sales markets were obtained by virus isolation and characterized by sequence analysis of the hypervariable and full length S1 glycoprotein gene. Ten out of 17 specimens were 90- 92% identical to the US isolates, CA/1737/04 and DMV/5642/06. The remaining 7 samples were 87-100% identical to the GA08 genotype. Data from phylogenetic analysis and virus neutralization will be presented. Significance of domestic poultry as a carrier of IBV will be discussed.

11184

Recombination in Avian Gamma-Coronavirus Infectious Bronchitis Virus

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The University Of Georgia: Poultry Diagnostic And Research Center

Recombination among coronaviruses has been well documented and studies have shown that recombination can result in the emergence of novel avian coronaviruses. Because the emergence of these novel viruses has a major impact on the poultry industry worldwide, we wanted to characterize the extent and frequency of recombination found in the avian coronaviruses. In this study, the genomes of select IBV strains were sequenced and analyzed for recombination along with other IBV strains, including vaccine strains. Evidence of recombination was found in every sequence analyzed. Genome location and frequency of recombination events will be presented and the significance discussed.

11185

Evaluation of Possible Interference between Arkansas and Massachusetts Vaccine Serotypes

Eunice N. Ndegwa, Samantha Bartlett, Vicky L. van Santen
Auburn University

Factors responsible for high frequency isolation of Ark-type infectious bronchitis virus (IBV) from commercial flocks in the southeastern U.S. despite the routine use of Ark-serotype IBV vaccines remain unclear. Although several factors could be responsible, one possible factor already demonstrated in human and veterinary medicine is serotype interference during simultaneous multiple serotype vaccine administration. This may occur if the co-administered vaccine strains replicate in the same tissues and one strain is hotter , hence receptor competition resulting in replication advantage of the hotter over the milder strain. Our recent finding that only minor viral subpopulations within the Ark-serotype vaccines are able to efficiently replicate in chickens suggests that these Ark serotype vaccines might be outcompeted by other serotype vaccines. We evaluated replication of Ark vaccine viruses in the presence of Mass vaccine viruses in 10- day old SPF chickens. Using an RFLP assay designed to differentiate between Mass and Ark vaccine viruses, we found that no Ark was detectable 3 and 6 DPV in tears of chickens vaccinated with combined Mass and Ark vaccines. Ark vaccine viruses could not be detected in most chickens in this group even when a highly sensitive Ark-specific RT-PCR was used, despite detection in control chickens inoculated with Ark vaccine alone by both RFLP and Ark-specific RT-PCR assays. Only Mass vaccine was passed on to non-vaccinated contact birds. These results point to a possible negative interference of replication of Ark serotype by the Mass serotype vaccine viruses when these vaccines are co-administered.

11186

Interactions Between Multivalent Attenuated Live Infectious Bronchitis Virus Vaccines in 1-Day-Old Chickens

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It is common practice to deliver multiple serotypes of live attenuated IBV vaccines to commercial broilers in the hatchery. However, it is extremely difficult to determine if the vaccines equally infect the birds because quantitative diagnostic tests that simultaneously detect each vaccine type are not available. In this study, we used our newly developed multiplex microsphere-based assay, which is capable of detecting each of the vaccine viruses in a single sample, to determine the interactions of IBV vaccines given to one-day old chicks. In addition, to access the immunogenicity of the multivalent vaccines, we examined the immune response to each of the vaccine viruses given to the birds.

11187

The Pathogenicity of Avian Metapneumovirus Subtype C (aMPV/C) Isolates from Wild Birds in Domestic Turkeys

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Avian metapneumovirus subtype C (aMPV/C) causes severe respiratory disease in turkeys. Previous report revealed the presence of aMPV/C in wild birds in the southeast regions of the U.S. In this study, aMPV/C positive oral swabs from Canada geese (CG) and American coot (AC) were passaged three times in the respiratory tract of SPF turkeys and used as p3 inoculum in the subsequent studies. Three-day-old commercial or SPF turkeys were inoculated oculonasally with wild bird aMPV/C p3 isolates. At 5 and 7 days post inoculation (DPI), severe clinical signs were observed in both of the CG and AC virus-exposed groups. Viral RNA was detected in tracheal swabs by RT-PCR at 3 and 5 DPI. In addition, immunohistochemistry showed virus replication in the nasal turbinate and trachea. All virus exposed turkeys developed positive antibody response by 14 DPI. Our data demonstrate that aMPV/C wild bird isolates induced typical aMPV/C disease in the domestic turkeys.

Reverse genetic studies of avian paramyxovirus type-3

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Avian Paramyxovirus (APMV) serotype 3 is one of the nine serotypes of APMV that infect a variety of avian species around the world. In chickens and turkeys, APMV-3 causes respiratory illness and drop in egg production. To understand the molecular characteristics of APMV-3, the complete genome sequences of prototype strain Netherlands and strain Wisconsin were determined. The genome length of APMV-3 strain Netherlands is 16,272 and for strain Wisconsin is 16,181 nucleotides (nt). Each genome consists of six non-overlapping genes in the order $^{3'}$N-P/V/W-M-F-HN-L$^{5'}$ similar to most APMVs. Comparison of the APMV-3 strain Wisconsin nt and the aggregate predicted amino acid (aa) sequences with those of APMV-3 strain Netherlands revealed 67 and 78% identity, respectively. The phylogenetic and serological analyses of APMV-3 strains Netherlands and Wisconsin indicated the existence of two subgroups within the same serotype. Both the strains were found to be avirulent for chickens by mean death time and intracerebral pathogenicity index test.

To further study the molecular biology and pathogenesis of APMV-3, a reverse genetics system for strain Netherlands was developed. The recovered recombinant virus showed *in vitro* growth characteristics and *in vivo* pathogenicity similar to those of parental virus. A recombinant APMV-3 expressing enhanced green fluorescent protein was also recovered, suggesting its potential use as a vaccine vector. Furthermore, generation and characterization of recombinant APMV-3 expressing Newcastle disease virus (NDV) F and HN proteins demonstrated that the F protein plays a major role in protection against virulent NDV challenge. Overall, the study

conducted here has several downstream applications. The complete genome sequence of APMV-3 is useful in designing diagnostic reagents and in epidemiological studies. The reverse genetics system for APMV-3 would be of considerable use for introducing defined mutations into the genome of this virus and developing a vaccine vector for animal and human pathogens.

11189

Replication of Recombinant Herpesvirus of Turkey Expressing Genes of Infectious Laryngotracheitis Virus (LT-rHVT) Following In Ovo and Subcutaneous Vaccination

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Replication of a recombinant herpesvirus of turkey vaccine expressing infectious laryngotracheitis virus genes (LT-rHVT) in specific pathogen free (SPF) and commercial broiler chickens after various vaccination protocols (amniotic route at 18 days of embryonation, ED; intraembryonic route at 19 ED; and subcutaneous at day of age, SC) was evaluated. Our results demonstrated that LT-rHVT replicated in the chicken after in ovo and SC administration but expression of the LTV antigen in the lung was low, being even lower in broiler chickens with maternal antibodies against HVT and LTV. Studies evaluating the mucosal immune response elicited by LT-rHVT in chicken with and without maternal antibodies are warranted.

11190

Field Experiences of the Hatchery Subcutaneous HVT + ILT Recombinant (INNOVAX) Vaccine in Table Egg Layers

Hugo A. Medina
Sparboa Farms, Inc

Field experiences of the hatchery subcutaneous HVT + ILT Recombinant (INNOVAX) vaccine in Table Egg Layers during production. We found some areas not previously discuss on the potential negative or challenges after the use of this Innovax vaccine. We needed to adjust our vaccination programs and procedures to minimized the presents on field challenges.

Comparative analysis of infectious laryngotracheitis virus (ILTV) live-attenuated vaccines and virulent isolates genomes

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Field evidence indicates that most of the ILTV outbreaks are caused by virulent strains closely related to the live attenuated vaccines. Early experimental evidence demonstrated that chicken embryo origin (CEO) vaccine significantly increased in virulence as a result of bird-to-bird passages, while the tissue culture origin (TCO) vaccine produced mild signs of disease. The objective of this study is to compare full genome sequences of the 1rst and 20th passages of the CEO and TCO vaccines in chickens with current virulent field isolates previously genotyped as CEO or TCO in an attempt to identify molecular determinants associated with ILTV virulence. Analysis of the CEO and TCO isolates subsets identified genes and genome regions that maybe related to increased virulence. Genome changes in the CEO subset as compared to CEO like Group V isolate genome yield 27 genome changes, while comparison of the TCO subset of isolates compared to the USDA strain provided 90 genome changes. Future work will resolve coverage of genome homopolymer stretches and will include a TCO virulent field isolate for comparison with the TCO subset to increase the resolution of the analysis to identify genome changes associated with an increase of virulence.

Management Procedures Used to Control ILT on Commercial Broiler Farms

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Infectious laryngotracheitis (ILT) can cause respiratory reactions in commercial broilers, which results in lowered body weight and increased processing plant condemnations. ILT viruses (V) isolated from these outbreaks are mostly chicken embryo (CEO) derived vaccines as determined by molecular techniques. These CEO vaccine viruses are difficult to inactivate and may readily infect newly placed flocks with an increased virulence after continual cycling in susceptible flocks. Conventional disinfection of houses including removal and replacement of litter is not always economically feasible, plus the use of recombinant ILT vaccines as of yet has not totally eliminated all ILT problems. Work at Auburn University has developed and tested the following management procedures for the reduction of increased virulent ILT vaccines from broiler farms: heating the house to 100 degrees F for 24 hours; use of poultry litter treatment; windrow (in-house) composting of litter; reduction of beetles and rats and use of biofilm elliminating water santizers. These techniques are now commonly used on commercial farms, because they do not require expensive litter changes and disposal of used litter, which may result in environmental pollution of ground water supply.

11195

CONSTRUCTION OF RECOMBINANT NEWCASTLE DISEASE VIRUSES, LASOTA STRAIN, EXPRESSING THE G PROTEIN OF AVIAN METAPNEUMOVIRUS, SUBTYPE A, B, OR C, FOR USE AS BIVALENT VACCINES

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Using reverse genetics technology, Newcastle disease virus (NDV) LaSota strain-based recombinant viruses were engineered to express the glycoprotein (G) of avian metapneumovirus (aMPV), subtype A, B, or C, as bivalent vaccines. Viral growth characteristics, such as 50% egg infectious dose (EID_{50}), 50% tissue culture infectious dose ($TCID_{50}$), hemagglutination (HA) end-point assays, and growth curves, of each recombinant virus was measured in vitro and compared to the lentigenic NDV LaSota wild-type virus. The mean death times (MDT) in chicken embryos and intracerebral pathogenicity index ratios (ICPI) pathogenicity in one-day-old chickens assays were also measured for each recombinant virus and compared to the wild-type virus. The antigenicity of these recombinant viruses was also evaluated in turkeys, the natural hosts of the aMPV viruses, which protected the turkeys against wild-type, velogenic NDV, CA02 strain, challenge. The results from these experiments suggest that the recombinant viruses may be safe vaccine candidates.

11196

Multiyear Surveillance of Avian Influenza on Wild Waterfowl on the Texas Coast

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We report on the first multiyear study of avian influenza viruses (AIV) on waterfowl wintering grounds of the Central Flyway, a historically understudied area of North America. A total of 7,478 cloacal swabs were collected over five years (2005 2006: 1,460; 2006 2007: 2,171; 2007 2008: 2,424; 2008 2009: 768; 2009 2010: 656) from 30 potential avian host species. Most samples (85.2%) were from dabbling ducks (genus Anas), while diving ducks (genus Aythya) accounted for 7.8%, and geese (genera Anser, Chen, and Branta) 3.2% of the samples tested. Waterfowl (Anatidae) comprised 98.8% of samples, with 2.0% from non-migratory dabbling ducks (genus Anas). All samples were screened for avian influenza virus (AIV) by AIV-matrix real-time RT-PCR (rRT-PCR); all rRT-PCR positive samples (607) were processed for virus isolation as well as 4,473 rRT-PCR negative samples. Differences were observed in apparent prevalence estimates over the four years between virus isolation (0.5, 1.3, 3.9, 0.7 and 2.6%) and rRT-PCR (5.9, 6.5, 11.2, 5.5 and 9.9%). We isolated 155 AIVs, of which two were obtained from rRT-PCR negative samples. All of the AIV subtypes we identified are common in North America; H3N8 and H4N6 were the most common subtype combinations isolated. We found no significant difference in AIV infection based on host sex, but did find that juveniles were more likely to be positive for AIV than adults. We also document that dabbling ducks were more likely to be positive for AIV than diving ducks, although not all dabbling ducks are equally likely to be positive.

11197

SURVEILLANCE OF AVIAN INFLUENZA VIRUS IN GRENADA, WEST INDIES

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The zoonotic potential of the circulating influenza subtypes in avian species underscore the importance of surveillance of influenza virus in the avian population. So far there is only one published study in the Caribbean region on the presence of avian influenza virus (AIV) in Barbados. A screening approach based on blood and cloacal and tracheal swabs to study the prevalence of influenza A in the avian population of Grenada was carried out in 2009-2010. We collected 230 blood samples and 230 mixed tracheal and cloacal swabs from backyard chicken (143) ,ducks (45), turkey (10), guinea fowl (1) and pigeon (31). Samples were screened by RT-PCR, embryo inoculation and ELISA for AIV. Neither AIV RNA was found by RTPCR nor Virus could be isolated in embryonated eggs. Twenty seven blood samples were antibody positive. All the positive sera were from backyard chicken. We conclude that backyard chickens of Grenada were exposed to AIV at some point.

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11198

Selective Isolation of Avian Influenza Virus from Cloacal Samples Containing Mixed Infection of Avian Influenza and Newcastle Disease Viruses

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Avian influenza viruses (AIVs) are important zoonotic pathogens that can infect many species including humans and poultry. Surveillance of AIVs is very important process to track the circulating AIVs that are of epidemiological importance. Waterfowl are the main natural reservoir of all the 16 HA and 9 NA subtypes. Waterfowl can be coinfected with other viruses besides influenza A viruses of which paramyxoviruses were more frequently isolated from waterfowl. Of epidemiological importance, Newcastle disease virus (NDV) is an important avian paramyxovirus type-1 (APMV-1) that can be isolated from waterfowl. Diagnosis of AIVs is a problematic process especially if other non-AIV heamaglutinating viruses are present in the same sample. Previously, samples positive to NDV are not screened or further processed for AIV by HI and NI tests, and this might be due to that NDV can overgrow AIV and thus reduce AIV detection and isolation. In this study, we isolated AIV of different subtypes such as H1N1, H3N8, H4N1, H4N2, H4N6, H5N2, H2N2, H7N1, and H7N2 from samples that were positive to NDV after the treatment of these mixed infection samples with chicken NDV polyclonal antiserum followed by inoculation into ECEs to reduce or eliminate the NDV from AIV samples. The importance of the detection of AIV from NDV positive samples is that we can isolate more AIVs that will be epidemiologically important for the knowledge of influenza surveillance and so we will not miss AIV viruses that are present in such NDV positive samples.

11199

Evaluation of Primer and Probe Mismatches in Sensitivity of Select RRT-PCR Tests for Avian Influenza

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The recent outbreak of pH1N1 in animals highlighted an imperfection of the matrix Real-Time RT-PCR (RRT-PCR) that has become the primary screening test for avian and swine influenza viruses. Four mismatches in one primer resulted in an important loss of sensitivity in the test. Bioinformatics for the matrix RRT-PCR test shows the forward primer and probe are highly conserved for almost all influenza isolates. The reverse primer, however, has 6 sites with important sequence variation. Two mismatches in the reverse primer are not uncommon in avian influenza samples, and 3 mismatches are observed in some lineages of virus. As mentioned previously the pandemic H1N1 viruses have 4 mismatches. In experimental testing of different variant viruses, viruses with 3 and 4 mismatches in the reverse primer had decreases in test sensitivity of 3-4 logs, which is unacceptable. Modifications of the original test were developed to improve sensitivity for all viruses. Bioinformatics analysis of H5 and H7 viruses also show important sequence variations that can affect sensitivity. Because sensitivity and specificity of RRT-PCR tests are dependant on proper annealing of primers and probes, efforts need to continue to evaluate tests against circulating strains of virus to assure proper test performance.

11200

Evaluation of Neuraminidase (NA) Subtypes 1 and 2 ELISAs for Detection of Avian Influenza Vaccinated/Infected Poultry Using an NA Heterologous Vaccination Strategy

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The development of serological assays that allow the differentiation of vaccinated/infected and vaccinate/non-infected can aid in the control of AIV in vaccinated populations. In this study we evaluated the ability of N1-ELISA and N2-ELISA to identify infected broilers and turkeys. As compared to the neuraminidase inhibition (NI) assay, the sensitivity and specificity of N1-ELISA to detect H1N1 infected turkeys was 0.80 and 0.97 respectively, while the sensitivity and specificity of N2-ELISA to detect H5N2 infected broilers was 0.84 and 0.98 respectively. The N1-ELISA was able to identify 33% of H1N2vaccinated/H1N1infected turkeys and 33% of H5N2vaccinated/H5N1 infected broilers, while N2-ELISA was able to identify 50% of H1N1vaccinated/H1N2challenge turkeys and 50% of H5N1vaccinated/H5N2infected broilers. Overall, the N1-ELISA and N2-ELISA showed a lower sensitivity than NI, but screening of NA antibodies by ELISA was an effective flock assay to rapidly identify exposure to the challenge virus during in a DIVA vaccination strategy.

11201

Clinical, Pathological and Virological Investigations of H6 Low Pathogenic Avian Influenza Virus Field Infection in Turkeys

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A case of low pathogenic avian influenza occurring in a 81-days old turkey flock in France was investigated in April 2010. Clinical signs included respiratory distress and cough, leading to asphyxia and death of the birds. The daily mortality reached up to 0.5% of the birds during 25 days. The total mortality due to this outbreak was 3.7% of the females and 6% of the males. Gross lesions included systematically exsudative tracheitis, airsacculitis and pneumonia, as well as splenitis. Microscopic lesions of the trachea were mainly an admixture of focal areas of necrosis and squamous epithelial metaplasia with a necrotic fibrinous luminal exsudate. Fibrinous airsacculitis and peritonitis were also observed. Tracheal swabs were sampled on 20 birds at the first day of the outbreak, then 10 days later, so as to follow up the virological status of the birds. A H6 LPAI virus was detected by RT-PCR in all the samples, and isolated on embryonated eggs. To assess the evolution of the viral quasi-species, a deep sequencing of the whole viral genome was performed on a pool of swabs at day 1 and day 10 of the outbreak, using a GS 454 pyrosequencing platform: the major virological features are presented and discussed.

PATHOGENICITY OF REASSORTANT H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES IN DOMESTIC DUCKS

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The pathogenicity of H5N1 highly pathogenic avian influenza (HPAI) viruses in domestic ducks has increased over time. These changes in virulence have been reported with viruses from countries with high population of domestic ducks including Egypt. In order to understand which viral genes are contributing to the increase in virulence of H5N1 HPAI viruses in ducks, we used reverse genetics to generate single-gene reassortant viruses with genes from two Egyptian H5N1 HPAI viruses of different pathogenicity, A/ck/Egypt/9402-CLEVB213/2007 and A/ck/Egypt/08124S-NLQP/2008. Intranasal inoculation of two-week-old domestic Pekin ducks with the reassortant viruses demonstrated that exchanging the genes of the highly virulent A/ck/Egypt/08124S-NLQP/2008 virus individually into the less virulent A/ck/Egypt/9402-CLEVB213/2007 virus increased clinical signs but did not induced the high mortality that the 2008 virus produced in ducks. This indicates that more than one viral gene is involved in the pathogenicity of H5N1 HPAI Egyptian viruses in ducks.

11203

Expression and Distribution of Sialic Acid Receptors in Tissues of Wild Birds

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Avian influenza viruses (AIV) preferentially bind to receptors containing sialic acid linked to galactose by an α2,3 linkage (SAα2,3Gal), while most human influenza viruses have tropism for the α2,6 linkage. The objective of this study was to determine influenza A virus receptor expression and distribution in tissues of wild birds. Various bird species from different orders including Anseriformes, Charadriiformes, Columbiformes, Passeriformes, Falconiformes, Accipitriformes, Gruiformes, Psittaciformes and Ciconiiformes were analyzed for the presence of two avian influenza virus (AIV) receptors, SAα2,3Galβ1,4GlcNac and SAα2,3Galβ1,3GalNac using the lectins Maackia amurensis I (MAAI) and Maackia amurensis II (MAAII), respectively. The expression of the human influenza virus receptor, SAα2,6Gal, was also analyzed using the lectin Sambucus nigra (SNA). It was previously reported that duck AIV preferentially bind to SAα2,3Galβ1,3GalNac, while gull AIV prefer SAα2,3Galβ1,4GlcNac. Overall, species reported to be more susceptible to infection to AIV had higher expression of SAα2,3Gal in the respiratory and intestinal tracts than less susceptible species. Varying expression of SAα2,3Galβ1,4GlcNac and SAα2,3Galβ1,3GalNac was observed in tissues of wild birds. This suggests differences in susceptibility to infection to AIV with differences in receptor tropism based on the inner part of the oligosaccharide receptors. Various species also expressed human-type receptors in tissues, which suggest the ability of some wild birds to bind human influenza viruses.

11204

Pretreatment of Chickens with Interferon Alpha Reduces Morbidity and Virus Shedding Following Low Pathogenic Avian Influenza Infection

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Type I interferons, including interferon alpha (IFN-a), represent a first line of defense initiated by the innate immune response following viral infection. Production of IFN-a results in a general antiviral state which has been shown to decrease morbidity and mortality in mammalian models. In these studies, we determined the protective potential of IFN-a applied to poultry prior to exposure to an H6N2 strain (A/Chicken/California/ K0301417/2003) and H7N2 strain (A/Turkey/Virginia/4259/2002) low pathogenic AIV. Intranasal application with IFN-a prior to and during active AIV infection reduced clinical signs of disease in a dose dependent manner. In addition, the incidence of viral shedding and viral titers from oral swabs was significantly reduced in IFN-a treated birds. Taken together, these studies show that IFN-a can protect chickens from disease associated with low pathogenic AIV and reduce the risk of transmission through decreased shedding.

11205

Virus-specific Antibodies Interfere with Avian Influenza Infection in Peripheral Blood Mononuclear Leukocytes from Young or Aged Chickens

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Avian influenza virus (AIV) infection was examined in peripheral blood mononuclear leukocyte cultures (PBMC) that were collected from 1-day-old chicks or from 52-week-old chickens. Virus-specific antibodies were incubated with AIV to model maternal antibody interference in vitro. Interferon-α (IFN-α), interleukin-1β (IL-1β), toll-like receptor (TLR) 7, and major histocompatibility complex class 1 (MHC1) genes were measured 24 hours after A/Turkey/Wisconsin/1968 (H5N9) low pathogenic AIV infection. Avian influenza increased all genes in PBMC from 52-week-old chickens; however, TLR7 and MHC1 genes were lower in AIV infected PBMC from 1-day-old chicks. Virus-specific antibodies interfered with IL-1β more than IFN-α in AIV infected PBMC.

11206

Aerosol Vaccination of Chickens with Baculovirus Expressed Virus-Like Particles Induced Immune Response in Chickens

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Influenza A virus (IAV) vaccination of animals and humans is a powerful tool for prevention and control of infection and disease. Currently licensed vaccines are egg-based and delivered by injection which is labor intensive. As an alternative vaccine manufacturing method, baculovirus grown in insect cells cultures can produce high yields of virus-like particles (VLP) which contain viral proteins but lack genetic material, and thus unable to replicate. VLP vaccines have been shown to be highly immunogenic after parenteral application in mice, ferrets, and humans. The aim of this study was to assess influenza VLPs as aerosolized vaccine (AE) in chicken as vaccination strategy. VLP used in this study were composed either of IAV matrix protein 1 (M), neuraminidase (N), and hemagglutinin (H) from H5N1 AIV or HA and M alone (HNM-VLP or HM-VLP, respectively). Plethysymography was used to determine the respiratory parameters for the chickens and to calibrate a controlled VLP aerosol application dose. One-day-old SPF chickens were vaccinated twice 14 days apart. As control, chickens were also vaccinated via intranasal (IN) instillation and intramuscular (IM) injection. Serum samples were tested for the presence of neutralizing and HI antibodies, or by indirect ELISA using baculovirus expressed H5-Vietnam. Both VLPs induced seroconversion after IM application. Chickens given HNM-VLP seroconverted after IN but not aerosol vaccination. In contrast, HM-VLP induced a specific antibody response after AE but not after IN application. These data show for the first time that non-replicating influenza VLPs might be used for mass aerosol vaccination in chickens.

11207

Towards the Development of a Virosome-Based Vaccine Against Avian Influenza Virus

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Virosome-based vaccines have been used extensively in humans, especially against influenza. However, there are currently no virosome vaccines for control of avian influenza virus (AIV), in chickens. In the present work, we assessed the ability of fusion-active virosomes for elicitation of antibody- and cell-mediated immune responses in chickens. Furthermore, we asked whether combining interferon-gamma or CpG ODN with virosomes would enhance immunogenicity of the virosome-based vaccine, leading to a reduction in virus shedding in vaccinated and infected chickens. Virosomes prepared from H4N6 avian influenza virus were used alone or in combination with purified baculovirus expressed recombinant chicken IFN-g or CpG. Subsequent to vaccination, chickens were infected with H4N6 virus and at several time points, cloacal and oropharyngeal swab samples were taken and tested for the presence of influenza virus. All birds immunized with various virosome preparations seroconverted and birds receiving the adjuvanted virosome formulation with CpG had significantly higher HI antibody titers compared to birds that were immunized with virosome alone or virosome with recombinant IFN-g. Induction of higher cytokine response was also evident in the group that had received virosome+CpG compared to the group that received virosome+IFN-g or virosome alone. Importantly, the immunized birds shed less virus compared to those that were not vaccinated. This lower shedding was associated with higher expression of IFN-g and IFN-b in spleen and lungs of immunized chickens. In conclusion, we demonstrated that a virosome-based vaccine can confer immunity against AIV and this response is enhanced by incorporating CpG ODN into virosomes

11208

Determination of Efficacious Vaccine Seed Strains for Use Against Egyptian H5N1 Highly Pathogenic Avian Influenza Viruses through Antigenic Cartography and In Vivo Challenge Studies

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Since 2006, there have been reported outbreaks of H5N1 highly pathogenic avian influenza (HPAI) in vaccinated chickens in Africa and Asia. This study provides experimental data for selection of efficacious H5N1 vaccine seed strains against recently circulating strains of H5N1 HPAI viruses in Egypt. Initially, the serological responses of chickens vaccinated using commercially available vaccines (A/Vietnam/1203/04 and A/CK/Mexico/232/94) against HPAI were examined and low mean titers (30 GMT) to the challenge virus (A/ck/Egypt/CLEVB-HK213(9402NAMRU3)/2007 (H5N1)) were found for both vaccinated groups post-challenge. Mean serological titers to the vaccine virus were 91 GMT pre-challenge and 147 GMT post-challenge for both vaccinated groups, respectively. Another study was conducted and a vaccine made from a A/chicken/Egypt/CLEVB-HK213(9402NAMRU3)/2007 (H5N1) was found to decrease mortality. Post-challenge mean titers of 97 and 97 GMT were found to the challenge viruses (A/ck/Egypt/CLEVB-HK213(9402NAMRU3)/2007 (H5N1) and A/ck/Egypt/0831-NLQP/2008 (H5N1)), respectively. Antigenic cartography was used to select HPAI vaccine seed strains to test against challenge virus strains. Based on results from antigenic cartography, five vaccine seed strains (rgA/goose/Guangdong/1996 x PR8, A/ck/Mexico/232/1994 [H5N2], A/Ck/Egypt/06959-NLQP/2006, A/ck/Egypt/0865-NLQP/2008, and A/goose/Egypt/0920/2009) were selected for testing against two challenge viruses. In conclusion, studies combining both antigenic cartography and targeted in vivo challenge studies show promise for selecting the most efficacious vaccine seed stains.

11209

Virus versus vaccine: Variants of Highly Pathogenic Avian Influenza Virus H5N1 from Egypt

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Highly pathogenic avian influenza viruses (HPAIV) of subtype H5N1 pose a severe threat to poultry industries worldwide. In Egypt, the virus was introduced in 2006 possibly by wild migratory ducks and despite intensive control efforts, HPAIV H5N1 gained a foothold in the poultry population and is now considered endemic in poultry in Egypt. Besides a stamping out policy, vaccination was chosen as an auxiliary tool to control HPAIV in poultry. Since then several phylogenetically distinguishable, co-circulating virus lineages have developed, raising the question about the potency of commercial vaccines regarding emerging variants.

In the current study efficacy of four different inactivated whole H5 virus vaccines representing different lineages within subtype H5 were tested in chickens against HPAIV H5N1 challenge viruses currently co-circulating in Egypt and representing two antigenically widely distinct clusters, i.e., Â¿variantÂ¿ (clade 2.2.1var) and Â¿properÂ¿ (2.2.1pro) viruses. All vaccines induced clinical protection against challenge with a classic 2.2.1pro Egyptian strain. In contrast, when challenged with a variant strain, only chickens vaccinated with the homologous Egyptian clade 2.2.1var virus or an inactivated re-assorted HPAIV H5N1 strain (Re-5, clade 2.3) were protected. However, only the homologous virus induced near sterile immunity whereas chickens clinically protected after Re-5 vaccination shed virus at day two after infection indistinguishable to recipients of other vaccines.

In conclusion, silent virus spread and consecutive vaccine-driven evolution of HPAIV H5N1 pose evident risks of HPAIV vaccination. At this point it is unclear whether continuous surveillance, including antigenic characterization and challenge studies is sufficient to successfully control HPAIV H5N1 by vaccination.

Subjective Welfare Assessments in the Hatchery

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During the course of a welfare audit, the auditor must make a subjective assessment of the impact of handling practices. Because there are relatively few objective and verifiable criteria for handling young poultry in the hatchery, it is necessary for the auditor to develop an understanding and awareness of practices that are within the expected standard of care. As is typical for other farming operations, small hatcheries will have different standards for handling than large hatcheries. The use of mechanical handling equipment, conveyors, counters, and vaccination devices introduces additional opportunities to create real or perceived welfare deficiencies.

This presentation will delineate specific observations that a welfare auditor should make as part of the overall and subjective assessment of handling practices in the hatchery. The author will address issues including welfare assessments during incubation, mechanical separation, hand pulling, euthanasia (specifically by maceration), culling, sexing, dropping birds (intentional or unintentional), short term and long term modifications, chick quality evaluation (relative to welfare), training and records.

Effects of Salmonella Enteritidis bacterins vaccination on layers's protection and immune response

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In this study, we evaluated the protection conferred by two commercial killed Salmonella Enteritidis (SE) bacterins. Four groups of layer hens were vaccinated with two immunization schedules either at 12 and 18 wks or at 16 wks of age. The control group was injected with a saline solution. The layer hens were later inoculated per os with 2 x 109 CFU of SE PT4 strain either at 55 or 65 weeks of age. Serum IgG and mucosal IgA antibodies were measured with an in-house SE whole cell antigen ELISA. The phagocytosis, oxidative burst, splenic T and B cells populations were analyzed using flow cytometry. Clinical signs, mortality, fecal shedding, egg yolks contamination and organ invasion by SE were assessed to evaluate vaccine protection. Potential horizontal transmission from inoculated laying hens to non-inoculated laying hens, housed in the same isolator unit, was also evaluated. In this experiment, only one bacterin (A) administered twice reduced SE shedding rate in inoculated laying hens and their exposed cagemate. There was no relationship between high IgG level and low SE isolation rates in organs and egg yolks. Significantly higher mucosal IgA levels were observed at day 1 and 7 days post challenge in oviduct of all vaccinated groups (except the twice vaccinated Layermune group) compared to the control group. Humoral efficacy to protect from SE contamination of egg yolk was only observed in the vaccin A groups. These results suggest that these bacterins were able to confer only partial protection against this SE PT4 strain infection.

11269

Maximizing Desired Outcomes for Therapeutic Antimicrobials

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The use of all applications of antimicrobials in poultry medicine and production is currently under review by the U.S. Food and Drug Administration Center for Veterinary Medicine (FDA-CVM). When antimicrobials are used for therapy in poultry, the desired clinical response may be easier to achieve when the most important factors affecting the effectiveness of antimicrobials in poultry are carefully considered. This analysis will not only enhance the odds of maximizing the desired therapeutic outcome but will also help in ensuring that treatment regimens are cost effective while at the same time minimizing the risk of antimicrobial resistance development. With FDA-CVM s current thinking, poultry veterinarians are likely to play a very important role in all uses of antimicrobials in poultry medicine and production in the future, therefore, they must also understand very well all the factors and potential interactions that may affect the therapeutic efficacy of antimicrobials so that they are always used in the most judicious and effective way possible. This presentation will concentrate on the most important factors that must be analyzed when the decision to use an antimicrobial has been made by a poultry veterinarian in order to maximize the probability of a successful therapeutic outcome.

11270

Justifying longer duration uses of tetracyclines, penicillins and sulfonamides in feeds and water: a summary of US usage and resistance patterns

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Tetracyclines, penicillin and sulfonamides are used individually and in combination in feeds and water for most U.S. food-producing animals including swine. While longer-duration, subtherapeutic uses in feed have raised controversy based on resistance concerns, actual reported resistance patterns among bacteria from U.S. slaughter animals and humans has been stable (even declining) after 12 years of testing. This study summarizes indications for use, as well as resistance trends as reported by the NARMS program since 1996, and correlations (if any) to total sales of these agents as reported by AHI. A structured summary of U.S. FDA-approved indication mentions was conducted using an online database of approved animal drugs. Resistance surveillance data were obtained from CDC, USDA and FDA NARMS annual reports from 2007, and 2008 for (non-Typhi) Salmonella for resistance prevalence. Annual aggregated resistance levels for nontyphoid Salmonella associated with food-producing animals were calculated from the weighted average contributions from cattle, swine, turkey and chicken carcass isolates for years 1997-2008. The lack of increasing trends or correlations evident from the data suggests there is little evidence of approved, judicious uses of tetracyclines, penicillins or sulfonamide antimicrobials in swine or other food producing animals driving problematic resistance in humans.

11271

Method Validation for the Rapid Detection of Salmonella Enteritidis from poultry houses and eggs

Rocio Crespo, Devendra Shah
Ahfsl-waddl, Washington State University

This study will seek to examine the role of vertical transmission and the described lag period. We will also compare 3 methods of Campylobacter detection from environmental and organ samples – membrane filtration, enrichment culture and molecular detection by PCR. Hatching eggs will be obtained from a known Campylobacter coli and jejuni positive breeder flock, and hatched at a PDRC facility. Day old chicks will be culled and organ samples tested. Chicks will then be placed in a highly biosecure environment and grown to 46 days. Environmental samples will be obtained at multiple times per week, and batches of birds will be sacrificed regularly for organ culture and PCR.

Real Time Antibiotic Use in Broiler Production

Timothy S. Cummings
College of Veterinary Medicine
Mississippi State University

Antibiotic use in the poultry industry has been ongoing almost since their discovery. There have been concerns over the decades about whether this antibiotic use in poultry production is contributing to the growing antibiotic resistance problem in human medicine. In an effort to ban certain antibiotic usage practices in food animal production, certain advocacy groups have provided estimates as to antibiotic usage levels by the poultry industry, but these estimates have been deemed to be grossly inaccurate by those familiar with poultry industry practices. This presentation will focus on providing a real time estimate as to the actual amount of antibiotics (primarily focusing on the feed additive antibiotics) currently being used in broiler production, and will discuss the implications this may have on antibiotic resistance.

11273

Development of a Novel Polymer Plenum Floor for Broilers to Replace Litter and Reduce House/Environmental Ammonia

Mark A. Dekich, Jeannine Harter-Dennis
Avihome Llc, 510 Naylor Mill Mill Road, Salisbury, Md 21801

Approximately 9 billion broilers are raised in the U.S.A. annually producing 25 billion pounds of manure. Broiler house floors have several types of litter that is used to absorb the manure moisture. In the broiler houses ammonia is produced by natural chemical reaction and released which is detrimental to the natural environment, broiler health, and human health. With 5 years of engineering and testing, special flooring has been developed to replace the litter. The flooring is comprised of 18 in. X 18 in. interlocking squares made from injection molded polymers mounted on top of coned pegs to make a plenum. The squares have engineered holes that allow moisture in the manure to be wicked away from the manure on the flooring into the plenum to be carried away by normal house ventilation. In consequence, manure moisture on the top of the floor is extensively reduced and the pH of the manure is maintained below 8 which reduces ammonia production. The flooring system also seals the floor from the ground which prevents heat loss down and moisture migration up. With moisture and pH controlled a better environment was produced in the broiler house. Other observed benefits were reduced dust levels (PM2.5), lower coccidiosis cycling, no darkling beetles, and improved broiler growth rate/FCR. So, eliminating litter with the innovative plenum flooring reduces ammonia production thus impacting the natural environment, poultry health/animal welfare, and broiler production parameters positively. Design and testing will be presented.

Low inclusion of a blend of organic trace mineral (Zn, Cu, Fe, and Mn) to broiler chickens diet is able to regulate oxidative stress

Harold Echeverry[1*], Alexander Yitbarek[1], Peris Munyaka[1], MohammadAli Alizadeh[1], Gilberto Camelo-Jaimes[1], Pengqi Wang[1], Karmin O[12], and Juan C. Rodriguez-Lecompte[1]

[1]Departments of Animal Science and [2]Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada - R3T 2N2.

Lipid peroxidation can alter the membrane properties of cellular and subcellular organelles crucial for maintenance of normal cardiomyocyte function. Minerals such as Zn and Cu have been recognised as important trace minerals in restoring or maintaining the oxidant-antioxidant balance in blood and tissues. Particularly, deficiency of Zn has been associated with increased oxidative damage of cell membranes caused by free radicals. Concentration of Malondialdehyde (MDA), an indicator of lipid peroxidation in the plasma, has been reported to be reduced with the supplementation of Zn to broiler diets. Our goal was to study the effect of organic trace minerals (OTM, Bio-Plex minerals, Alltech Inc) inclusion to monensin based broiler diet on MDA, mortality and immunological parameters. Three dietary treatments (T1 = Negative Control; T2 = Bacitracin Methylen Salycylate supplemented diet and T3 = T2 + OTM; n=6) with 60 birds per pen and six pens per treatment were randomly assigned to observe the effect of OTM inclusion on the plasma MDA level. Birds were fed *ad libitum* for 42 days. Data was analyzed with the mixed procedure of SAS and means subjected to analysis of variance. A significantly higher level of plasma MDA as a result of supplementation of BMD in the diet (T2) was reversed with the inclusion of OTM to the diet (can you please include levels of MDA as per treatment affects that were statistically different by treatment). This observation was further corroborated with the fact that mortality rate in T3 was lower than that in T1 and T2. Furthermore, Bursa: body weight and spleen: body weights were not significantly different between the treatments.

11275

Sampling Unhatched Embryos As A Method Of Salmonella Detection In A Turkey Hatchery

Dave V Fernandez, David C Mills
Agforte

Fluff sampling is a common test used by turkey hatcheries for the detection of Salmonella in day old poults. The objective of the study is to evaluate sampling of unhatched embryos as an alternative test. A prospective cohort study involving eggs hatched from five turkey breeder flocks during a six month period will be conducted to compare fluff and unhatched embryo sampling procedures for the detection of salmonella in a turkey hatchery.

This Turkey Is Not A Sept/Tox; This Turkey Is Not A Cadaver: This Turkey Does Not Have TOC

Eric Gonder
Butterball Llc

After working with several different turkey processing plants operating under traditional, NITS and HIMP inspection regimes, it has become evident that many personnel responsible for inspection have some rather strange ideas regarding disposition of turkeys for condemnation or salvage procedures.

This will be a pictorial tutorial of what the author perceives to be appropriate dispositions of different turkey carcasses and some of the common inappropriate dispositions offered, hopefully with an opportunity for brief discussion.

Monitoring on Salmonella Infections in Turkey Flocks in Germany and European Union Control Measures

Hafez Mohamed Hafez
Institute Of Poultry Diseases, Free University Berlin

In present study commercial turkey flocks were monitored for salmonella between 2002 -2010. Two boot swabs samples were collected from each monitored flock 3 weeks prior to slaughtering and examined bacteriologically. The obtained results showed that a continuous reduction in the prevalence all salmonella serovars could be detected. In 2002 till 2004 strong reduction was observed from 18.1 % to 5 %. Between 2007 and 2010 the number of positive test flock varied between 3. 2% to 5.0% The Prevalence of S. Typhimurium showed similar reduction. S. Enteritidis could not be detected in all examined samples since 2006. The obtained results still reinforce the fact that it is essential and important to continue the efforts on reducing salmonella infections. With respect to the Council Regulation 2160/2003/EC (EC, 2003) on the control of salmonella and other specified food-borne zoonotic agents, starting from 12.12.2010 fresh poultry meat may not be placed on the market for human consumption when Salmonella was detected. The criterion laid down does not apply to fresh poultry meat destined for industrial heat treatment or another treatment to eliminate salmonella. Till now it is not clear what is meant by Salmonella Â¿ all serovars or only ST and SE? The EU-Regulations will be discussed.

11278

Highly pathogenic strains of Salmonella Enteritidis show enhanced tolerance to acid, oxidative stress and better survival in egg albumen.

Quincy L. Hawley, Carol Casavant, Tarek Addwebi, and Devendra H. Shah.
Nc State University Cvm

Despite decades of research, questions remain unanswered regarding the epidemiology of colonisation of broilers in the field. Particular controversy surrounds the potential role of vertical transmission. Birds are currently believed to become colonised from 2-3 weeks of age onwards.

11279

Osteomyelitis in Tom Turkeys with Green Discolored Livers

Hoffman, AL; Slater, M; Rives, D; Martin, MP; Barnes, H
North Carolina State University, Poultry Health Management

Turkeys with osteomyelitis develop a characteristic enlargement and green discoloration (GL) of their livers that is most obvious when the birds are processed and bled-out. Other inflammatory musculoskeletal lesions, e.g., arthritis, tendonitis, tenosynovitis, bursitis, etc. can also cause GL and are often associated with osteomyelitis. Presence of GL in a carcass at processing is used to identify carcasses with Turkey Osteomyelitis Complex (TOC). GL carcasses are cut and examined for inflammatory lesions and condemned if lesions are found. In previous studies, flocks in which GL was caused by TOC had osteomyelitis in the tibiotarsus that ranged from 40-60%. When TOC was not the cause of GL, the occurrence was 5-10%. Objectives of this study are to determine the number of GL carcasses in the initial 10-bird sample that have osteomyelitis in the proximal tibiotarsus, isolate and identify the bacteria responsible for osteomyelitis, and correlate the findings with % GL condemnations, weekly mortality, and basic production data. Presence of leg atrophy as an indicator of osteomyelitis was also looked at. Ten toms from each flock that exhibited GL had both of the proximal tibiotarsal bones examined. Bones with osteomyelitis were cultured. The study lasted for 18 weeks looking at toms from one company in the Southeastern United States. Thirty-six flocks and a total of 718 legs were examined.

11280

Analysis of changes in chicken gut microbial communities and metabolic potential in response to growth promoters

Timothy J. Johnson, Jessica L. Thorsness-Danzeisen, Hyeun Bum Kim, and Richard E. Isaacson
University Of Minnesota

Growth promoters have been used by the poultry industry for over fifty years as a means to increase bird health and feed efficiency. Despite their benefits, the precise mechanisms of growth-promoting antibiotics remain incompletely understood. Alternative approaches to reproduce their effects are critical to the success of poultry producers. The primary objectives of this study were to utilize deep pyrosequencing to assess changes in chicken gut microbial communities and metabolic potential in response to subtherapeutic antibiotic administration. Conventional broiler chickens were housed in research facilities at the University of Minnesota and fed 1) a control diet without any additives, 2) a diet containing monensin only, 3) a diet containing monensin and virginiamycin, or 4) a diet containing monensin and tylosin. Birds from each group were sacrificed at days 0, 7, 14, and 35 of the experiment and their cecal contents collected. Total DNA was isolated and subsequently used for sequencing of 16S rDNA and shotgun sequencing of total DNA. Sequences from each of the experimental groups were compared for differences taxonomical units and functional groups, and significant enrichments and depletions were observed in a number of taxonomic and metabolic functional groups assessed. The changes were often subtle, requiring deeper sequence analyses for their identification. Overall, these results suggest that the use of growth promoters in broiler chickens acts to mature the gastrointestinal microflora at a faster rate than feed lacking antibiotics. Future work will focus on the identification of alternative therapies that elicit similar avian gut microbial responses.

11281

Evaluation of Size Variation in Commercial Turkeys from a Single Breeder Flock Part II Bacterial and Viral Profile.

Daniel Karunakaran, David.V Rives, Jodi. Benson, Greg. Siragusa, J.M Day.
Danisco Animal Nutrition

Flock uniformity in commercial turkeys is a constant challenge. Day-old poults are very vulnerable to pathogenic challenges. Poult placements are often comprised of offspring from several breeder flocks of different ages and strains. Poult enteritis can result in stunted birds, further accentuating size differences. Close examination of relatively uniform flocks from a single breeder source and exhibiting no overt disease can also reveal considerable size variation.

This study looks at four commercial hen flocks placed within a three week period, all from a single breeder source flock. Three large and three small birds were selected from each flock at one, two, and three weeks of age. Each bird was weighed and a blood sample obtained before euthanasia by cervical dislocation. Intestinal samples were then obtained from each bird for histology, bacterial analysis, and viral analysis. Approximately fifty birds were weighed from each flock at five weeks of age.

To examine the impact of the intestinal microbiota of turkeys on growth rate and performance, the gut bacterial communities of high gaining birds (high) were compared to low gaining (low) from the same production house. The intestinal tracts from three high and three low poults were collected from each of four production houses at weeks 1, 2 and 3 of age. Genomic DNA was isolated separately from intestinal mucosa and cecal content after pooling. 16S rDNA was amplified and samples subjected to pyrosequencing

This information one day will lead to preventive measures and intervention strategies to maintain flock uniformity. Body weights, serology, and histology will be presented here. Results of histopathology, body weights and other performance indicators were presented by a pervious companion paper.

11282

A profile of Aspergillus spp. Dissemination into Broiler Hatching Egg Air Cells via In-Ovo Injection holes

Robert W. Keirs
Mississippi State University

In the US approximately 9 billion broiler hatching eggs annually receive in-ovo vaccination into their air cells at 18.5 and 19.5 days of hatch. This results into a patent needle hole subject to the entry of fungal spores, if present, in the ventilation supply. The most common and vivid example is seen in the air cell of otherwise uncontaminated unfertilized eggs having but a single or few punctiform centers of blue fungal vegetative growth. Their pattern and presence appears distributed consistently among embryonic ages. To profile this incidence, such fungal growth was incorporated into the Hatching Efficiency Analysis System (HEAS) by Keirs et al. The profile represents 84 flocks hatching in 84 separate machines on 5 hatch dates in 3 different months in the same hatchery and consists of over 8,000 hatching egg residue. The overall incidence of Aspergillus spp. was 6.35%. As a percent of their respective embryonic age the unfertilized and 3 day were 9.10 and 9.42%. As a present of the total incidence the same ages were 69.4% and 17.85% respectively. The incorporation of Aspergillus spp. incidence within HEAS adds to hatchery quality control monitoring.

An Essential Role of Avian Nod1 in Host Innate Immunity

S. Kim[1], C.L. Keeler[2], E.A. Wong[1], C.M. Cox[1], L.H. Sumners[1], and R.A. Dalloul[1]*
[1]Virginia Tech, Blacksburg, VA
[2]University of Delaware, Newark, DE

Pathogen recognition receptors (PRRs) are membrane-bound or intracellular molecules that stimulate host innate immunity upon recognition of microbial-associated molecular patterns. Nucleotide-binding oligomerization domain-containing protein 1 (Nod1) and 2 (Nod2) are major intracellular PRRs that induce innate immune responses through cytosolic recognition of bacterial components produced during the synthesis and/or degradation of peptidoglycans. In mammals, Nod1 is stimulated by γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP) from mostly Gram-negative and some Gram-positive bacteria, while Nod2 is stimulated by muramyl dipeptide (MDP) from both Gram-negative and Gram-positive bacteria. To date, the *Nod2* gene has not been identified in sequenced avian genomes including chicken, turkey, and zebrafinch. In this study, we describe the cloning and characterization of the avian Nod1 molecule, as well as its various ligands.

11284

Introduction and Dissemination of a "New" Salmonella Serotype Throughout a Commercial Turkey Company

Ronald L Lippert, Dale C Lauer
Willmar Poultry Company

The California – Minnesota Salmonella Agreement, also known as the Cooperative Program for the Control of Salmonella in Turkeys, began in 1979. This marked the beginning of routine Salmonella testing of Minnesota turkey breeder flocks. Since that time over 30,000 positive samples have been serotyped by the Minnesota Poultry Testing Laboratory (MPTL), with over 100 different serotypes identified. The original purpose of the program was to eliminate Salmonella types that caused disease in the progeny of infected turkey breeder flocks, with the primary focus Salmonella typhimurium. It was an animal health program focusing on the reduction of disease due to vertical transmission. In recent years food safety has been in the forefront of concerns for food producing companies. This has elevated the need for Salmonella monitoring beyond the historic “breeders only” approach. To that end, WPC established a comprehensive, coordinated Salmonella monitoring program in 2006. This monitoring program detected the introduction of a new serotype (one not isolated in the previous 30 years of routine breeder flock testing) in feed ingredients in June of 2008. This paper will demonstrate how this coordinated monitoring program was able to follow this “new serotype” through the system from breeder flock to the processing plant. This episode “validated” the WPC Salmonella monitoring program and revealed some “opportunities” to prevent future reoccurrence. It also increased the level of confidence that we may actually be able to demonstrate any beneficial effects of future interventions.

11285

Development of the Immune System in Broiler Breeder Pullets Receiving Various Vaccination Programs and Feeding Systems in Controlled and Field conditions

Enrique Montiel, Jeffrey Buhr, Nelson Cox, Beverly Wills, Charles Hofacre and Jeanna Wilson
Merial Select Inc

Broiler Breeder pullets from a single grandparent flock were vaccinated in ovo with Marek's vaccines HVT and SB1 or a Vector HVT and IBD vaccine. The birds were housed in an experimental broiler breeder facility at the University of Georgia. The birds were fed ad libitum for 2 weeks. At 2 weeks of age, the birds were divided in two groups and fed every day or using the Skip a Day program until light stimulation. A control group was placed in the field in a commercial pullet farm where they were fed using the Skip a Day system. All birds received a conventional live+inactivated broiler breeder pullet vaccination program where 3 different inactivated IBD vaccines were tested. The development of the immune system was measured by weekly bursa to body weight ratio, spleen weights and histopathology of the bursa, spleen and thymus between two and 17 weeks of age. Serum samples for antibody titers against Infectious bursal disease were collected every 4 weeks to assess B cell function. Progenies from all experimental groups were challenged at 14 days of age with pathogenic IBDV virus isolates to evaluate maternal antibody transmission.

11286

Detection of natural Campylobacter colonisation in experimentally reared broiler chickens from a positive commercial breeder flock.

Peter M. O'Kane, Stephan G. Thayer, César G. Wilsmann, Denise L. Brinson, Rodrigo A. Espinosa, Nelson A. Cox, Roy Berghaus, Margie D. Lee, Bwalya Lungu, Charles L Hofacre

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

Campylobacter infection continues to be a leading cause of bacterial gastroenteritis in people. FSIS regulations for Campylobacter reduction may soon be implemented in the USA.

Despite decades of research, questions remain unanswered regarding the epidemiology of colonisation of broilers in the field. Particular controversy surrounds the potential role of vertical transmission. Birds are currently believed to become colonised from 2-3 weeks of age onwards.

This study will seek to examine the role of vertical transmission and the described lag period. We will also compare 3 methods of Campylobacter detection from environmental and organ samples – membrane filtration, enrichment culture and molecular detection by PCR. Hatching eggs will be obtained from a known Campylobacter coli and jejuni positive breeder flock, and hatched at a PDRC facility. Day old chicks will be culled and organ samples tested. Chicks will then be placed in a highly biosecure environment and grown to 46 days. Environmental samples will be obtained at multiple times per week, and batches of birds will be sacrificed regularly for organ culture and PCR.

11287

Effects of Whole House Brooding on Broiler Performance and Footpad Dermatitis Prevalence

Edgar O. Oviedo-Rondón, Michael J. Wineland, Marcelo R. Dalmagro, Julian Ruiz, Daniel Ruiz, Diego García Valencia, and Martina Pérez Serrano
Department Of Poultry Science, North Carolina State University, Raleigh, Nc, 27695

Broiler growers normally conduct brooding in a section of the house and gradually provide more space to the flock as broilers grow. A delay to provide adequate space to the flock causes increased humidity in the brooding area, litter caking, worsening of air quality, and reduction in space for broilers to have access to feed and water. This mismanagement normally last for two or three days during the second week of life; however, its detrimental effects have important impacts at processing age on flock uniformity, final body weights (BW), leg problems, foot pad dermatitis (FPD), and potentially other health issues. Three experiments under commercial conditions were conducted to evaluate the effects of the practice of whole house brooding (WHB) in comparison to the traditional partial (1/3) house brooding (PHB). Day old chickens of similar strain, age of breeder flock, and incubation conditions were placed. Individual BW were recorded for 250 broilers per treatment at 21 and 56 days. Leg health issues, and gait scores were evaluated at 56 days of age. Feed consumption and mortality were recorded per house to calculate feed conversions. Data of gas usage, carcass yield, carcass condemnations, FPD prevalence, and general profitability were collected to evaluate the feasibility of implementing WHB. Initial results indicated that broiler flocks WHB may have similar average BW and FCR, have lower FPD prevalence, better uniformity, and fewer condemnations than flocks PHB; however the additional energy cost to heat the house can offset the benefits of WHB under current market conditions.

11288

Rapid and cost-effective molecular Salmonella serotyping assay utilizing Luminex® multiplexing technology.

Gunjot S. Rana, Brad Mire, Douglas Waltman, Michaela R. Hoffmeyer
Luminex Corp.

Salmonella infections are among the leading bacterial cause of illness in the United States, with shell eggs being the primary source of human infections. In 2004 there were 1,376,514 cases of Salmonella with 14,264 hospitalizations and 427 deaths. Egg shells are primarily infected through the environment and the transovarian route. Because of health concerns and the cost burden associated with Salmonella infections, regulations require reporting of Salmonella serotypes for all detected cases. Traditionally Salmonella serotyping has been done manually by tube agglutination. This process is time consuming, subjective and expensive. We propose a rapid, molecular method capable of completely serotyping 85% of the 100 most common serotypes in 3.5 hours while providing partial results for most other serovars. The advantages of using a molecular approach include ability to serotype rough and problematic isolates, improved reliability, high throughput, increased time efficiency, decreased cost, all while yielding results that mirror traditional serotyping methods. The assay was tested on samples obtained from the Georgia Poultry Lab Network and results were compared to classical agglutination. Samples with discrepant results were tested by NVSL. These results demonstrate that the molecular serotyping assay is an accurate and rapid alternative to traditional serotyping. Adoption of this method will lead to decreased serotyping cost for egg and poultry producers and increased ability to control outbreaks.

Evaluation of Size Variation in Commercial Turkeys from a Single Breeder Flock Part I

David V. Rives, John K. Rosenberger, Oscar J. Fletcher, Dan Karunakaran, Greg R. Siragusa, J. Michael Day
Prestage Farms, Inc., Po Box 438, Clinton, Nc 28329

Flock uniformity in commercial turkeys is a constant challenge. Poult placements are often comprised of offspring from several breeder flocks of different ages and strains. Poult enteritis can result in stunted birds, further accentuating size differences. Close examination of relatively uniform flocks from a single breeder source and exhibiting no overt disease can also reveal considerable size variation.

This study looks at four commercial hen flocks placed within a three week period, all from a single breeder source flock. Three large and three small birds were selected from each flock at one, two, and three weeks of age. Each bird was weighed and a blood sample obtained before euthanasia by cervical dislocation. Intestinal samples were then obtained from each bird for histology, bacterial analysis, and viral analysis. Approximately fifty birds were weighed from each flock at five weeks of age.

Evaluation of body weights, serology, and histology from each sampling period will be presented here along with flock performance data. Results of bacterial and viral analysis are to be presented in a companion paper.

11290

Relative Performance in Rural Uganda of Two Breeds of Scavenging Backyard Chickens

Jagdev M. Sharma, Hope R. Mwesigye, D. K. N. Semambo, Esau Galunkande, Diamond Musinga, Tonny Aliro and Sylvia L. Sharma
Arizona State University

Indigenous chickens raised in small backyard village flocks in Africa constitute over 80% of the total poultry production in the continent. Often, eggs and meat derived from these small flocks owned by individual families are the main source of income and animal protein for the household. An average Indigenous hen lays 20-40 eggs per year and the male reaches a maximum live weight of approximately 1.5 kg. This rate of performance does not meet the economic or nutritional needs of the household. We examined the potential of introducing a dual purpose, high performance hybrid chicken, Kuroiler in rural household in Uganda. In an initial trial, the relative performance of Indigenous chickens and Kuroilers, raised under identical management conditions, was compared. The observations included weight-gain in males and females, egg production, mortality, serologic response to vaccines and incidence of infectious diseases endemic in the area. At 14 weeks of age, the duration of the trial to date, the relative body weight values were as follows: Birds in village scavenging conditions: Kuroiler male 1448g, Indigenous male 768g, Kuroiler female 1279g, Indigenous female 754g. Birds in confinement with access to feed: Kuroiler male 2550g, Indigenous male 1808g, Kuroiler female 2108g, Indigenous female 1436g. Data on egg laying and other parameters will be available within the next several months. The mortality thus far has been minimal in both breeds. The preliminary data on weight gain and survival indicate that Kuroiler chickens thrive as scavengers in African rural environment and perform far better than the Indigenous chickens. Introduction of Kuroilers in rural Uganda and possibly other parts of Africa is likely to substantially improve nutritional and economic benefits of backyard flocks for village households.

11291

Implementation of MSRV Methodology for Salmonella Monitoring-Customer Service and Economic Effects

Kevin W. Smith
Georgia Poultry Laboratory Network

This presentation will dicuss our implementation of a new MSRV methodology for Salmonella detection in poultry environmental samples. Specific data will be shown to demonstrate the positive impact of the change on turnaround time, labor, and cost savings.

11292

Influence of incubation temperature on turkey poult intestinal development and susceptibility to poult enteritis

Jennifer R. Sottosanti, James S. Guy, Frank W. Pierson, Rami A. Dalloul, Audrey P. McElroy

Department Of Animal And Poultry Sciences, Virginia Tech, Blacksburg, Virginia 24061

Early mortality, grow-out performance, and health status of commercial turkeys can be largely impacted by exposure to stressors during early development, potentially including temperature stress during incubation. Non-optimal incubation conditions may detrimentally affect intestinal and immune system development and maturation, thereby impacting digestion, absorption, and immune function and the ability of the poult to respond to challenges during the first several weeks of life. Turkey coronavirus (TCV) is a viral inducer of poult enteritis, and has been shown to elicit damaging effects on gastrointestinal structure and function, immune organ development, and overall immunocompetency. There may be a relationship between poult enteritis severity and gastrointestinal tract maturation. Thus, stressors that contribute to delayed tissue maturation or hinder organ function could increase poult susceptibility to enteritis. The objectives of this study were to evaluate the effects of incubation temperature stress during the plateau phase of embryonic development (ED24-28) on turkey poult intestinal and immune system development and susceptibility to poult enteritis up to 21 d post-hatch based on measurements of intestinal development, maturation, and functionality, immune organ development, peripheral blood profiles, antibody response to TCV, and bird performance.

AAAP/AVMA Future Annual Convention dates and locations

Future Convention Sites

2012	August 4 - 7	San Diego, CA	San Diego Convention Center
2013	July 20 - 23	Chicago, IL	McCormick Place, West Building
2014	July 26 - 29	Denver, CO	Colorado Convention Center

Other Meetings of Interest

XVII Congress of the World Vetertinary Poultry Association, August 14-18, 20011

Future Trends in Animal Agriculture Symposium 9/21/2011

8th Internationl Symposium on Avian Influenza to be held in London, UK at the Roral Holloway April 2012

Development of a transfer plasmid for expression of foreign genes in meleagrid herpesvirus type 1

Stephen J. Spatz and Laszlo Zsak
Southeast Poultry Laboratory, 934 College Station Road, Athens Ga 30605

Current research indicates that meleagrid herpesvirus type 1 (MeHV-1) is an excellent vector for the expression of avian immunogens. Classical methods using marker rescue approaches are often time consuming and require the inclusion of undesirable additional genetic material (antibiotic resistant, green fluorescent protein, etc). Generation of vaccine candidates using the recombination machinery of E. coli would elevate these problems. We have developed a transfer plasmid which contains MeHV-1 sequences for homologous recombination into the thymidine kinase locus, a strong eukaryotic promoter upstream of a multiple cloning site. The object of this study is to clone a chicken codon-optimized gene encoding VP-2 of chicken parvovirus into this transfer plasmid, insert the VP-2 gene within the genome of a MeHV-1 bacterial artificial chromosome (BAC) construct and reconstitute the virus upon transfection of chick embryo fibroblast.

Genetic Characterization of a Vaccine strain of Fowlpox virus

Deoki N. Tripathy, Bahaa Fadl-Alla and Francisco Robles
University Of Illinois

Vaccination against fowlpox has been done by the poultry industry for over 80 years. However, in recent years several outbreaks of fowlpox have occurred in previously vaccinated chicken flocks indicating lack of adequate protection by some fowlpox virus vaccines. Molecular examination of fowlpox virus isolates from such outbreaks reveals the insertion of full-length reticuloendotheliosis virus (REV) in fowlpox virus genome. The field strains of fowlpox virus containing full-length REV show variable pathogenicity and some are more virulent than others. REV is associated with immunosuppression and tumor formation. Additionally, fowlpox virus persists in the environment for extended periods. In this regard, presence of A-type inclusion body gene and photolyase gene in fowlpox virus genome protect it from environmental insults and assist in its prolonged survival in the environment. Considering that genetic evaluation is important to select an appropriate strain of fowlpox virus for vaccination, in this study a vaccine strain of fowlpox virus was genetically characterized by PCR amplification of selected genes e. g. thymidine kinase, photolyase, 39K, A-type inclusion body, hemagglutinin, epidermal growth factor and REV provirus. Nucleotide sequence of the PCR amplified products of the virus genome were sequenced and compared with the available sequences. Results indicate that the genome of the vaccine strain has high degree of similarity to the US strain of fowlpox virus and lacks REV provirus.

The Use of peGFP *E. coli* to Establish that Yolk Sac Infection Occurs Via the Broiler Chick Navel

Ana M. Ulmer-Franco, Lynn M. McMullen, Gaylene M. Fasenko
Department of Agricultural, Food and Nutritional Science, Poultry Research Centre, University of Alberta, Edmonton, AB, Canada, T6G2P5

Escherichia coli infections are a common cause of hatchling morbidity and mortality. Omphalitis/yolk sac (YS) infection, produced by avian pathogenic *E. coli (APEC),* is the main cause of chick mortality during the first week post-hatching. Improper navel healing at hatching has been associated with poor chick performance and hypothesized to be directly related to YS infections. Our objectives were to: 1) determine whether *E. coli* carrying a plasmid for enhanced green fluorescent protein (peGFP) could be used to prove that bacterial infection occurs via the chick navel into the yolk sac; 2) establish if a lack of navel healing promotes the infiltration of peGFP *E. coli* into the YS. One APEC strain (EC234) was transformed to carry the peGFP and used to infect the navel area of newly hatched broiler chicks. In 3 replicate experiments chicks were selected based on the presence of a healed/closed (H) or unhealed/open (U) navel. Over 5 consecutive days, the skin peripheral to the navel and the YS of hatchlings were collected, prepared for fluorescence microscopy, and examined for the presence of fluorescent *E. coli*. Navel health had a significant effect on the presence of peGFP *E. coli*; a greater percentage of U chicks than H chicks were positive for fluorescent bacteria (51.5% vs. 36.0%). This experiment provides evidence that unhealed navels do serve as a route for bacteria entry into the chick. It also offers the possibility of using fluorescence-tagged bacteria to track the route of infections.

11296

Development of a Biosecurity Training Program for Poultry Catch Crews and Drivers

Eva A. Wallner-Pendleton DVM, MS, DACPV[1], R. Michael Hulet, PhD[1], Gregory Martin, PhD[1], Paul Patterson, PhD[1], Chrislyn Wood, DVM[2] and Lindsay Harlow, BS[2].
[1]Pennsylvania State University
[2] USDA

Most poultry companies conduct detailed training programs for employees on animal welfare and biosecurity. However, there are a number of independent contract companies involved in handling and transportation of poultry that do not have formalized training for their employees. Often these crews and transporters travel widely in a given region and serve multiple companies and farms. A training and certification program was developed that targets these groups. This program covers animal behavior and welfare, biosecurity and disease recognician, herding techniques, employee safety and emergency procedures.

The development of this third party training and certification program for handling and transportation crews in both English and Spanish is discussed. Results and evaluation of the initial trainings will be covered.

Relationship between Salmonella culture findings from poultry and their environmment.

Doug Waltman
Georgia Poultry Laboratory Network

The decision of a broiler breeder company to qualify for the NPIP U.S. Salmonella Enteritidis Clean classification provided an opportunity to compare the results of pullorum-typhoid serology, bird culture and environmental sampling for Salmonella.

11298

Generation and evaluation of a LaSota strain-based recombinant Newcastle disease virus (NDV) expressing the glycoprotein (G) of avian metapneumovirus subgroup C (aMPV-C) as a bivalent vaccine

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To develop a bivalent vaccine, a recombinant Newcastle disease virus was generated by using the NDV LaSota strain with insertion of the G gene of aMPV-C. The biological assessments of the recombinant virus, rLS/aMPV-CG, by conducting the mean death time, intracerebral pathogenicity index, and growth dynamics assays showed that rLS/aMPV-CG was slightly attenuated compared to the wild-type LoSota strain. The expression of aMPV-C G protein in rLS/aMPV-CG-infected Vero cells was detected by immunofluorescence assay. Vaccination of turkeys with rLS/aMPV-CG provided protection against NDV and aMPV-C challenges. The results suggest that this recombinant virus is a safe and potential bivalent vaccine candidate.