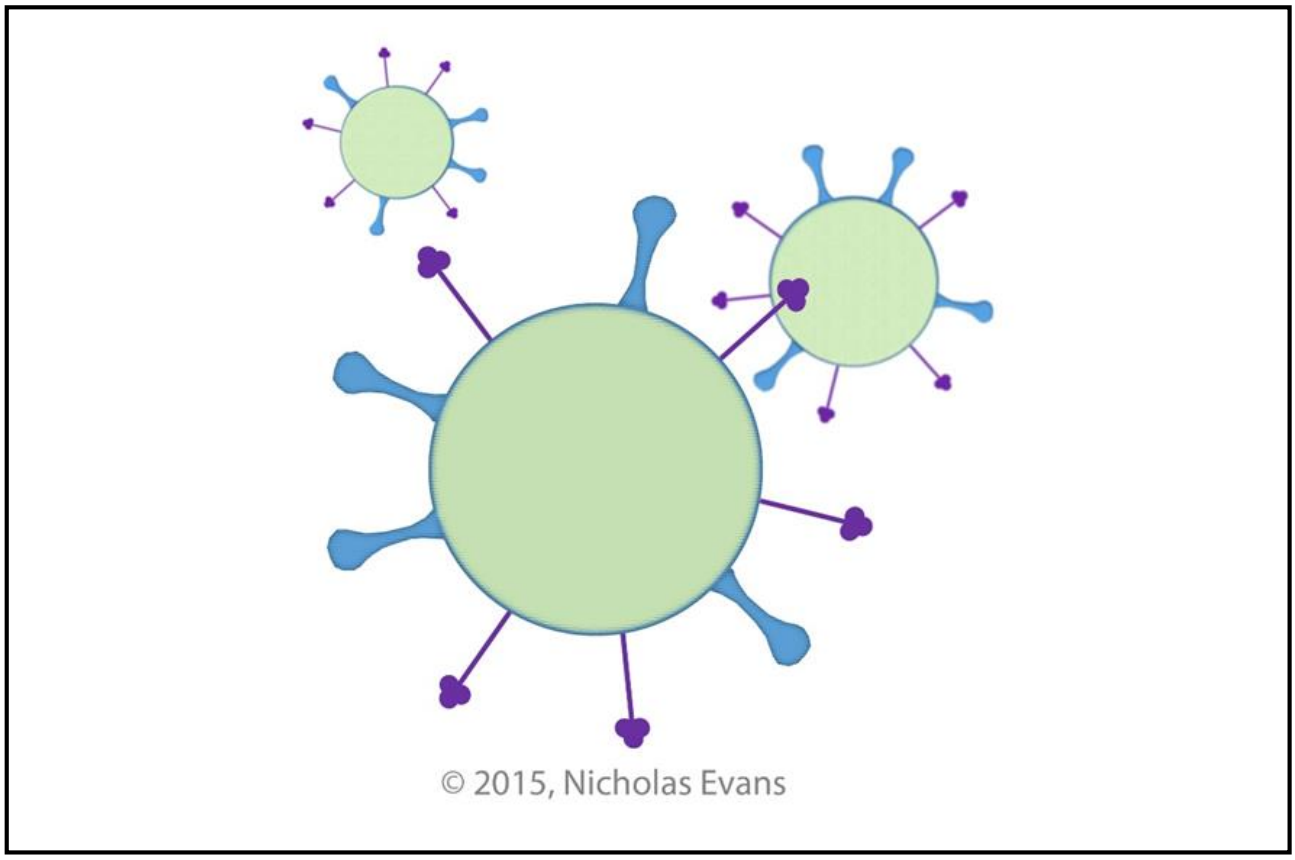
Proceedings of the 87th Northeastern Conference on Avian Diseases

Symposium: Control & Prevention of Avian Influenza



September 16, 2015
State College, Pennsylvania

VirginiaTech
VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY

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September 2015

Dear Attendees,

I am honored to be serving as the 87th Northeastern Conference on Avian Diseases (NECAD) Chair and look forward to an informative meeting. This past year, highly pathogenic avian influenza (HPAI) has swept across western and mid-western states. This outbreak has resulted in the loss of approximately 50 million birds and is the most costly food-animal disease outbreak in U.S. history. Migratory birds are believed to have carried and spread the virus, first to backyard flocks and then to commercial flocks. Since mid-June no new poultry flocks have been identified as infected with HPAI. However, as wild birds begin moving south for the winter, our concern is that once again we will be faced with an HPAI outbreak. To that end, this year's Symposium will address control and prevention measures for HPAI.

Please also join us for the NECAD Scientific Session following the Symposium. The Scientific Session has served has a platform for the presentation and discussion of research in all areas of poultry heath. Your questions and input are greatly appreciated and help to guide future research.

We hope the Symposium and research presentations are insightful and provide an opportunity to share new ideas.

Sincerely,

Nicholas Evans

Nicholas Evans, PhD
Chair / Secretary, 87th NECAD

87th Northeastern Conference on Avian Diseases

2015 Pennsylvania Poultry Sales and Service Meeting

Continuing Education Certificate

This certifies that

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was in attendance and qualifies for up to 10.0 contact hours of continuing education held on September 16-17, 2015, State College, Pennsylvania

Nicholas Evans, PhD
Chair and Secretary, 87th NECAD

NECAD Symposium – Control & Prevention of Avian Influenza

September 16, 2015

8:00 Welcome

8:10 Eric Benson, The Highly Pathogenic Avian Influenza Virus Outbreak of 2015: Lessons Learned in Depopulation and Disposal.

8:50 Justin Brown, Understanding the Epidemiology of Avian Influenza in Wild Birds.

9:30 Klaus-Peter Behr, AI-Preparedness in Germany: Concepts for Monitoring and Depopulation.

10:10 Break

10:40 Darrell Kapczynski, Research Update on H5 HPAI in the U.S.

11:20 Fidel Hegngi, The United States of America (USA) 2014/2015 HPAI – Lessons Learned, Biosecurity, Indemnity, and Plans for the Fall.

12:15 Lunch Buffet

NECAD Scientific Session

September 16, 2015

1:30 Anita Menconi, Field Experience on the Use of Probiotics in Chickens and Turkeys.

1:45 David Collins*, Evaluation of Avi-Lution® for the Reduction of *Salmonella* in Poultry by Quantitative Bioluminescence Imaging.

2:00 Richard K. Gast, Microbiological Consequences of Different Housing Systems for Laying Hens: Field and Experimental Infection Studies.

2:15 Ashli Moore, Neural Regulation of Broodiness in Turkeys.

2:30 Hassan Mahsoub*, Update: Characterization of Cellular Receptors for Turkey Hemorrhagic Enteritis Virus on Target B Cells.

2:45 Paul H. Patterson, Ensiling Poultry Carcasses for Biosecure Preservation and Virus Destruction.

3:00 Break

3:30 Yin-Ting Yeh, An Integrated Microfluidic Device for Rapid Avian Influenza Virus Capture and Detection.

3:45 Yi Tang, Whole Genome Alignment Based One-step Real-time RT-PCR for Universal Detection of All Serotypes and Genotypes of Avian Orthoreoviruses.

4:00 Huaguang Lu, Next-Generation Sequencing (NGS) for Full Genome Sequencing of Newly Emerging Avian Reovirus Field Strains and Other Viruses.

4:15 Utsav Pandey*, DNA from Dust: the First Field-Isolated Genomes of MDV-1, from Virions in Poultry Dust and Chicken Feather Follicles.

4:30 David Kennedy, Survey of Marek's Disease Virus Concentration across Poultry Farms in Central Pennsylvania, USA.

4:45 Student Award and NECAD Business Meeting

5:00 Adjourn

* Denotes an individual eligible for “Best Student Presentation”

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NECAD Symposium

Wednesday
September 16, 2015

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The Highly Pathogenic Avian Influenza Virus Outbreak of 2015: Lessons Learned in Depopulation and Disposal

Eric R. Benson[1] and Robert L. Alphin[2]

[1]Professor, Department of Animal and Food Sciences, University of Delaware, Newark, DE

[2]Instructor, Department of Animal and Food Sciences, University of Delaware, Newark, DE

The Spring 2015 H5N2 outbreak in the upper Midwest was the most extensive and costly animal health emergency in U.S. history. The major steps involved in an emergency poultry disease outbreak response include surveillance, quarantine, depopulation, disposal, and disinfection. Biosecurity and scene control was a factor in disease spread. Common mass depopulation methods include carbon dioxide or water based foam treatment. Depopulation and disposal of floor reared poultry was adequate, but caged layer hens presented a particular challenge for both major steps.

Understanding the Epidemiology of Avian Influenza in Wild Birds

Justin D. Brown[1], Kyle Van Why[2], and David Stallknecht[3]

[1]Pennsylvania Game Commission, Pennsylvania State University, Animal Diagnostic Laboratory, Wiley Lane, State College, PA

[2]United States Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, Harrisburg, PA

[3]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, Department of Population Health, The University of Georgia, 589 D. W. Brooks Drive, Athens, GA

Certain groups of wild birds represent the primordial reservoir for avian influenza (AI) viruses. Although AI viruses have been detected in a wide diversity of avian species, most isolations have been from waterfowl, gulls, or shorebirds. Research over the last 50 years has defined our existing understanding on AI ecology within the wild bird reservoir system. While there are still many questions that remain unanswered, there are distinct epidemiologic patterns of AI virus infection in wild birds that are critical for understanding mechanisms and risks for spillover into poultry or other aberrant hosts. Historically, highly pathogenic (HP) AI viruses have uncommonly been detected in wild birds and the H5 HPAI virus outbreaks experienced in North American during 2014-2015 challenged several aspects of the AI paradigm. The focus of this talk will be on discussing what is historically known about AI in the wild bird reservoir system and in what ways the recent H5 HPAI viruses are unique.

AI-Preparedness in Germany: Concepts for Monitoring and Depopulation

Dr. Klaus-Peter Behr

AniCon Labor GmbH, Hoeltinghausen, Germany www.anicon.eu

In Germany laboratory testing for avian influenza is obligatory for defined suspicious cases including rising mortality or sudden decreases of food- and / or water-consumption or egg-laying. Many private veterinary labs as well as corporate industry labs are running PCR-tests based on test kits licensed and released by the federal government. Almost any AI-case over the last years has been detected first by private labs and then immediately checked and mostly confirmed by governmental laboratories.

The turkey- and duck industries since many years are monitoring every flock at slaughter by serology for AI-antibodies on a voluntary basis, thereby gaining a continuous picture of the situation in the country especially regarding upcoming LPAI-situations without any clinical symptoms.

Although the government is also running monitoring systems in game birds, especially game waterfowl, we never found any situation where findings in game birds were followed by infections with the same AI-type in domestic poultry in proximity to such findings. Nevertheless, AI in domestic poultry, especially in free-range farms, occurs more often in areas close to roosting places of wild waterfowl.

Earliest Detection, close and quick communication between the private sector and the authorities, immediate culling decisions by the authorities and quick and clean depopulation are the tools which need to go coinstantaneous for a successful limiting of AI-spread especially in poultry-dense areas. A broadminded compensation system for flocks hit by culling decisions is absolutely essential to guarantee the cooperation of the animal owners.

With this concept, we were able to stop a quick-spreading H5N3-LPAI-outbreak in turkeys during winter 2008/2009. 45 out of > 200 farms in the area had to be depopulated. Based on PCR-results culling-decisions were made immediately and > 80 % of these flocks did not develop any seroconversion until culling, meaning that we were able to detect and depopulate these flocks within less than 8 to 10 days post infection.

Based on the experience especially during HPAI in the Netherlands (2003) and LPAI in Germany (2008/2009) staff, machinery and equipment seem to play the most important role in AI-spreading, much more than air-borne transmission.

The AniCon-Group with about 100 staff is a company dedicated to veterinary concepts for the livestock industries and involved in early disease detection (AniCon Labor GmbH) by developing and marketing licensed PCR-kits (Kylt® AI-PCR www.kylt.eu) as well as developing and running depopulation concepts (AniCon Vorsorge GmbH).

Concepts for the immediate and quick depopulation of poultry farms need to combine the targets of:

1) working safety, especially protection against zoonotic agents;
2) animal welfare;
3) minimizing the risk of disease spread and

4) economical aspects.

Based on these targets, the most important principles for our depopulation concepts are:
1) minimizing the number of staff involved
2) depopulation without handling live birds
3) depopulation without moving live birds outside of poultry barns

By minimizing the number of staff involved we are decreasing the number of staff exposed to AI-virus in case of zoonotic virus types and we are also decreasing the number of staff potentially supporting AI spread, even if unintended. Minimizing staff means using methods which do not need individual handling of live birds. This can be done primarily by whole house gassing with CO_2, it can also be done by driving birds into transport containers, which are then moved into mobile containerized gassing units.

If we need to handle birds individually, this will lead to a severe increase of staff needed and – even worse – will need professional loading crews, because only these crews are experienced to handle the birds. Loading crews normally do not want to work in AI-culling as they take the risk to lose their other customers with healthy birds. Even if paid for a 3-day quarantine of such crews after the culling, we see a tremendous risk of disease spread if such crews are involved in culling activities.

To decrease the risk of disease spread by staff, we developed mobile showering units.

The aforementioned arguments led us to a cascade of methods for culling where whole-house gassing with CO_2 (in chickens and turkeys – not used in waterfowl) is the preferred method, followed by the use of containerized gassing units run with either CO_2 or Ar-CO_2, this again followed by the use of mobile electrical water bath systems.

The mobile locker and showering units for staff , the mobile cleaning and disinfection-systems for trucks, the different mobile culling systems, their capacities as well as their pros and cons will be demonstrated and discussed.

Research Update on H5 HPAI in the U.S

Darrell R. Kapczynski

Southeast Poultry Research Laboratory, USDA-ARS, Athens, Georgia

In December 2014, following the outbreak of H5N2 highly pathogenic avian influenza (HPAI) in British Columbia, Canada, near-simultaneous isolations of HPAI virus (HPAIV) were recovered from the northern pintail (NOPT) duck and captive gyrfalcons in Washington State. Two different HPAIV subtypes were identified in these birds, H5N2 in NOPT and H5N8 in gyrfalcon. In January 2015, additional HPAIV isolations were reported in California, Idaho, Oregon, Nevada, Utah and Washington. These H5 isolations were confirmed in wild birds, backyard poultry and a commercial turkey operation. During this time period an additional HPAIV subtype, H5N1, was also reported in a green winged teal in Washington State.

Sequence analysis of these HPAI viruses has confirmed that all are related to a H5N8 HPAIV isolated in South Korea in January 2014. These H5N8 HPAI viruses evolved from the Asian H5N1 HPAI-lineage that was first recovered in Guangdong, China, in 1996. These H5N8 viruses belong to Asian-H5 clade 2.3.4.4 and contained only Eurasian gene segments. Subsequently in November 2014, the H5N8 viruses were detected in commercial turkeys, chickens and ducks in Germany, Netherlands and United Kingdom, respectively. The detection of H5N8 in Western Europe implicates migratory wild birds as likely source of virus spread out of Asia.

In North America, the H5 HPAI viruses spread quickly along migratory waterfowl pathways, including the Pacific, Central and Mississippi flyways. The H5N2 and H5N8 viruses were soon detected in wild birds and commercial poultry in many Midwest states resulting in unprecedented losses. The majority of field outbreaks occurred during a three month span between April, May and June. To date, approximately 50 million birds have died or been depopulated, such that there is need and interest in developing vaccines against these viruses. Protective immunity against HPAI largely depends on the development of an antibody response against a specific subtype of challenge virus. Historically, the use of antigenically closely matched isolates has proven efficacious when used as inactivated vaccines. More recently, the use of recombinant live AI vaccines expressing a hemagglutinin (HA) gene from an individual isolate have proven effective against multiple lineages of HPAI. The focus of this presentation will be to provide an overview of research into these viruses, with an emphasis on vaccine research for control of disease.

The United States of America (USA) 2014/2015 HPAI – Lessons Learned, Biosecurity, Indemnity, and Plans for the Fall

Dr. Fidelis (Fidel) N. Hegngi

Senior Staff Veterinarian – Avian Health

Avian, Swine and Aquatic Animal Health Center, Surveillance, Preparedness and Response Services (SPRS), Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), 4700 River Road, Unit 46, Riverdale, MD 20737-1231

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USDA, APHIS, VS, Mission

The USDA, APHIS, VS, protects and improves the health, quality, and marketability of our nation's animals and animal products, and veterinary biologics by: preventing, controlling, and/or eliminating animal diseases, and monitoring and promoting animal health and productivity, and licensing veterinary biological products intended for use in the treatment or diagnosis of diseases in animals.

USDA AI Prevention and Control Program

The goals of the AI prevention and control program are to: (1) quickly diagnose, control, and prevent the spread of all H5 and H7 AI subtypes; (2) improve biosecurity, sanitation, and disease control in commercial poultry, live bird marketing system (LBMS) and high-risk poultry sectors (auctions, small sales, flea markets, swap meets, farmers markets, feed stores, botanicas, and backyard or hobby flocks); and (3) minimize the effects of AI on the U.S. LBMS and commercial poultry industry.

The USDA, APHIS, VS national AI surveillance plan has five objectives: (1) Rapidly detect H5/H7 AI in domestic poultry populations; (2) Ensure that low pathogenicity H5/H7 AI strains are not circulating in poultry populations where they may spread and mutate into high pathogenic avian influenza (HPAI) viruses; (3) Provide consistency with international surveillance guidelines for trade purposes; (4) Protect public health through early detection and control of H5/H7 AI viruses; and (5) Demonstrate to trading partners and consumers that U.S. poultry is free of potentially dangerous influenza viruses.

Current Status of U.S. HPAI Outbreak

Since December 2014, the USDA, APHIS and the U.S. Department of the Interior (DOI), The U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) have detected HPAI H5 viruses in U.S. domestic poultry (backyard and commercial flocks), captive wild birds, and wild birds. These novel HPAI viruses were introduced by migratory waterfowl, and subsequently spread rapidly along 3 of the 4 North American migratory Flyways (the Pacific,

Central and Mississippi Flyways). There have been a total of 232 affected premises (21 backyard flocks; 211 commercial flocks) as of June 18, 2015. Commercial premises with HPAI have been found in nine States: MN-109, IA-71, SD-10, WI-9, NE-5, CA-2, MO-2, ND-2, and AR-1. Approximately 50 million commercial birds (7.5 million turkeys and 42.1 million chickens) have been affected and depopulated. The depopulation losses represent 10 percent of U.S. average table egg layer inventory, 6.33 percent of U.S. average pullet inventory and 7.46 percent of U.S. average turkey inventory. The USDA, industry leaders, and State veterinarians have worked diligently to stop the spread of this disease. No human cases of these HPAI H5 viruses have been detected in the United States. This resulted in the largest disease outbreak that the Federal government has ever responded to. We have deployed more than 700 USDA employees, some of them multiple times, and nearly 3000 contractors. To date, the outbreak has cost about $700 million, and we are expecting it will reach $1 billion before we are finished assuming that there are no new cases.

My presentation will focus on the 2014/2015 U.S. HPAI outbreak Lessons Learned, Biosecurity, Indemnity, and Plans for the fall.

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NECAD Scientific Session

Wednesday
September 16, 2015

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Field Experience on the Use of Probiotics in Chickens and Turkeys

Anita Menconi and James Barton

Pacific Vet Group, Fayetteville, AR

Probiotics have been used for several years in an attempt to improve intestinal health. Recently, microbial products have been extensively studied as supportive treatments for raising poultry without antibiotics. Regardless of the intended use, when testing a probiotic product efficacy, effects on commercial flocks are an essential consideration. Therefore, our objective was to analyze the effects of commercial probiotics on poultry health and performance under field condition. Selected field application of three different probiotic products was evaluated. The probiotic products tested were a hatchery applied lactic acid bacteria probiotic, FloraStart®, a water administered lactic acid bacteria probiotic, FloraMax-B11®, and a feed administered *Bacillus subtilis* spore direct fed microbial, Sporulin®. Four field experiments were conducted to evaluate the impact of FloraStart® on seven days mortality and body weight of commercial broiler chicks. In experiments 1 and 2, a decrease in the 7 days cumulative mortality was observed in the houses where chicks received probiotic in the hatchery. In experiments 3 and 4, chicks sprayed with the probiotic product at the hatchery showed significant ($P < 0.05$) higher body weight at 7 days of age compared to controls. Three field experiments were conducted to evaluate the influence of FloraMax-B11® on performance and mortality of commercial broilers and turkeys. In experiment 1, FloraMax-B11® was administered to turkey hens at feed changes. Significant ($P < 0.05$) increase on average daily gain and market body weight as well as improvement in feed conversion was observed. In experiment 2, FloraMax-B11® was administered to broilers at 2, 11, and 22 days. Increase on average daily gain and market body weight and significant ($P < 0.05$) decrease in feed conversion and final mortality was observed. In experiment 3, two broiler flocks (Control and FloraMax-B11® at days 8, 21, and 35) were compared at processing. FloraMax-B11® treated broilers showed significant ($P < 0.05$) improvement on body weight, feed conversion, and mortality compared to control. Moreover, the association of one dose of FloraStart® in the hatchery and one dose of FloraMax-B11® in the field showed improvement of body weight, mortality, and condemnation of broilers at market age. Additionally, two field experiments were conducted to evaluate the impact of Sporulin® on performance, mortality, and *Salmonella* spp. reduction. In experiment 1, Sporulin® in the feed significantly ($P < 0.05$) improved body weight gain and reduced *Salmonella* spp. count in cecal contents. In experiment 2, Sporulin® in the feed reduced cumulative flock mortality and improved feed conversion and body weight in broilers. Generally, commercial field trials differ from controlled research trials by several aspects such as higher variability, including issues with blocking and measurement assessment. Due to this, it is essential to test and analyze probiotic products under both controlled and commercial conditions in order to evaluate and validate their effects. Overall, the discussed probiotic products showed improvement on health and performance of chickens and turkeys under field condition, which correlated with research trials previously performed.

Evaluation of Avi-Lution® for the Reduction of *Salmonella* in Poultry by Quantitative Bioluminescence Imaging

David A. Collins[1], Hassan Mahsoub[2], Nammalwar Sriranganathan[2], William F. Pierson[1], Rami A. Dalloul[3], Michael E. Persia[3], Nicholas P. Evans[1]

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[3]Department of Animal and Poultry Sciences, College of Agriculture and Life Sciences, Blacksburg, VA

The gastrointestinal microbial flora plays an important role in the overall health and well-being of poultry. Prior to the establishment of such flora, poultry are more susceptible to developing persistent *Salmonella* infections. These infections may contribute to increased risk of human salmonellosis through product contamination. Consequently, there is a need for control measures that reduce *Salmonella* carriage levels in poultry, including the use of direct-fed microbials (DFM). The objective of this study was to determine the efficacy of Avi-Lution®, a DFM product developed by Agri-King, Inc., for the reduction of *Salmonella* in turkey poults.

Day-of-hatch turkeys were either spray treated according to the manufacturer's directions (≈ 1 gram/100 hatchlings) with a single dose of Avi-Lution® (ST), spray treated with a single dose and provided Avi-Lution® ad-libitum (drinking water; 30 grams Avi-Lution®/125 gallons of water) for the duration of the study (STAL), or left untreated (positive and negative controls). Birds were orally gavaged with 10^8 colony forming units of bioluminescent *Salmonella* Typhimurium (ST, STAL, and positive control). Gastrointestinal tissue samples were collected at 4 hours, 1 day, 4 days, 8 days, and 12 days post-inoculation (PI) for bioluminescence imaging (Meckel's diverticulum to cloaca) and most probable number (MPN) enumeration (ceca).

This study was analyzed as a factorial ANOVA (time x treatment). Although no interaction between time and treatment was observed for bioluminescence, both main effects were significant. Slice analysis indicated differences at 4 and 8 days PI which was followed by a two-way contrast analysis. At 4 days PI, the STAL treated group, but not the ST treated group, had significantly reduced bioluminescence compared to the positive control. At 8 days PI, both STAL and ST treated groups had significantly reduced bioluminescence compared to the positive control. Similarly, there was no interaction between time and treatment for the MPN enumeration, but both main effects were again significant. The slice analysis indicated that the differences were at 4 and 12 days PI. Two-way contrast revealed that the STAL treated group, but not the ST treated group, had significantly reduced MPN values compared to the positive control (4 and 12 days PI). This investigation indicates a decrease in *Salmonella* carriage in response to early Avi-Lution® treatment in poultry. Utilizing new research methodologies such as bioluminescence imaging can provide a better understanding of infection dynamics to ultimately improve flock health and solve post-harvest food safety concerns.

Microbiological Consequences of Different Housing Systems for Laying Hens: Field and Experimental Infection Studies

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

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[2]Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC

[3]Department of Animal Science, Michigan State University, East Lansing, MI

A significant proportion of human illnesses caused by *Salmonella* are linked to the consumption of contaminated eggs. In response, substantial government and private industry resources are committed to comprehensive *Salmonella* testing and risk reduction programs for commercial egg-laying flocks. Environmental conditions in poultry housing facilities directly affect opportunities for *Salmonella* introduction, transmission, and persistence. Many important environmental influences are determined by the various production housing systems used in the modern commercial egg industry. A thorough assessment of the public health implications of different housing systems for egg-laying flocks depends on understanding both how they influence the environmental survival and spread of *Salmonella*, and how they affect the course and outcome of infection in hens.

In a large field study, environmental swabs and egg shells pools were collected from commercial laying hens housed in conventional cages, enriched colony cages, or cage-free aviaries. These samples were tested for the numbers of total aerobes and coliforms, and for the presence of *Salmonella*. Environmental aerobe and coliform counts were highest for aviary drag swabs (7.5 and 4.0 log cfu/ml, respectively) and enriched colony cage scratch pad swabs (6.8 and 3.8 log cfu/ml). Aviary floor and system wire shell pools had the greatest levels of aerobe contamination in egg shell pools (4.9 and 4.1 log cfu/ml). Hens from all housing systems were shedding *Salmonella* spp. (89-100% of manure belt scraper blade swabs). There were no significant differences between the housing systems in *Salmonella* detection from either environmental samples or egg shells. These results indicate that each housing system has unique microbiological risk factors and associated management challenges for pathogen control.

In a series of experimental infection studies, laying hens housed in either conventional cages or enriched colony cages were orally inoculated with *Salmonella* Enteritidis (SE). Significantly more hens in conventional cages shed SE in their feces during the first 4 weeks post-inoculation than did hens in enriched colony cages (43% vs. 29%) in 1 study, but the frequency of horizontal transmission of infection was not affected by housing type in a 2nd study. Likewise, although the frequency of SE invasion to internal organs in a 3rd study was significantly greater among hens in conventional cages than in enriched colony cages (93% vs. 53% of spleens; 25% vs. 10% of ovaries), hens from the 2 housing systems laid internally contaminated eggs at similar frequencies (3.6 – 4.0%) in the 4th study. Accordingly, although parameters which differ between conventional and enriched cages (including stocking density and behavioral restriction) may affect some aspects of susceptibility to SE colonization, this influence does not extend to all aspects of infection or product contamination.

Neural Regulation of Broodiness in Turkeys

Ashli F. Moore[1,2], Vincent M. Cassone[2], and Paul A. Bartell[1]

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[2]Department of Biology, College of Arts and Sciences, University of Kentucky, Lexington, KY

Egg production in turkeys is regulated by photoperiod. Long days stimulate egg production, followed by broodiness and a photorefractory stage in which long days are no longer stimulatory and egg production ceases. Understanding the photoperiodic regulation of reproduction is critical for developing strategies to increase production efficiency. Recent studies have implicated a specific area of the brain, the premammillary nucleus of the hypothalamus (PMM), as the site of photoperiodic timekeeping. Our research seeks to clarify the role of the PMM in photoperiodic regulation of egg production in turkeys. We are examining the effects of PMM lesions on egg production in order to test the hypothesis that this brain area mediates the development of photorefractoriness in hens. Sexually mature hens (Nicholas 500), held on short photoperiods (8:16 light:dark), were divided into two groups: a photostimulated group (n=45), and an unstimulated group (n=45). Birds in the photostimulated group were transferred to long photoperiods (16:8 light:dark) prior to PMM lesion surgery. Egg production was monitored by trap nesting, and photostimulation was confirmed in individual birds prior to surgery. Birds in the unstimulated group received PMM lesions while still being held on short photoperiods, prior to photostimulation. Following surgery, birds in all groups were held on long, photostimulatory photoperiods.

The photostimulated group will allow us to determine whether or not the PMM plays a role in regulating broodiness and/or photorefractoriness, because these birds were already laying eggs prior to PMM lesion. The unstimulated group will allow us to determine whether or not the PMM plays a role in regulating the photostimulation of egg production, as these birds received PMM lesion prior to photostimulation. Egg production is monitored by trap nesting: 32 trap nest boxes are checked every 2-3 hours over the course of the 16-hour day and individual egg laying recorded. Data are collected on the number of eggs laid for individual birds, as well as broody behaviors (going into nest boxes in the absence of egg production). We are currently monitoring egg production and will continue to do so for a total of 6 months, or until birds cease laying eggs. Data will be analyzed for total egg production, weekly egg laying frequency, latency to initiate lay following photostimulation, daily nesting frequency, and latency for initiation of broodiness and/or photorefractoriness. After monitoring egg production, brains will be histologically examined to qualify the extent of PMM damage. Results from this study will illuminate the role of the PMM in the photoperiodic control of egg production.

Update: Characterization of Cellular Receptors for Turkey Hemorrhagic Enteritis Virus on Target B Cells

Hassan M. Mahsoub[1, 2], Nicholas P. Evans[1], and F. William Pierson[1]

[1]Department of Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

[2]Department of Poultry Production, Alexandria University, Alexandria, Egypt

Turkey hemorrhagic enteritis virus (THEV) is a member of the genus *Siadenovirus* which is so named because of the presence of an unusual sialidase homolog in the viral genome. THEV is known to specifically target B-lymphocytes in turkeys. The cell surface receptors facilitating viral attachment / internalization (A/I) have not yet been characterized. For our studies, a B-lymphoblastoid cell line (MDTC-RP19) commonly used to produce cell culture vaccines was employed as the viral-specific target cell. Surface adenovirus receptors are often comprised of terminal sialic acid (SA), carbohydrate, and/or polypeptide moieties. To identify those components necessary for A/I of THEV, RP19 cells were pre-treated with a number of reagents including neuraminidases (NAs) and lectins to cleave and block SAs, respectively, sodium periodate to oxidize carbohydrates, and proteases. Controls remained untreated. Cells were then centrifuged, rinsed 3 times, and re-suspended in media containing THEV. After 1 hour of incubation (time sufficient only for A/I), cells were centrifuged, rinsed twice, and lysed (freeze-thaw). The effect on A/I was measured by determining residual virus genome copy number (vGCN) using real-time PCR. Previous experiments on NAs and lectins showed little effects on A/I due to incubating the treated cells under suboptimal conditions. Under newly optimized conditions, preliminary data showed that cleaving SA by NAs partially inhibited A/I. This indicates the role of surface SAs in virus attachment to target cells. Furthermore, vGCN was reduced by the mild treatment of cells with sodium periodate and proteases. This suggests that THEV requires both carbohydrate and polypeptide surface structures for A/I. Receptor proteins were further characterized using a virus overlay protein binding assay (VOPBA). Preliminary results showed that THEV binds to 2 proteins having approximate molecular weights of 69 kDa and 85 kDa. Further work to confirm and characterize the protein molecules that bind to THEV is ongoing.

Ensiling Poultry Carcasses for Biosecure Preservation and Virus Destruction

Paul H. Patterson[1], Mike Hulet[1], Patricia Dunn[2], Huaguang Lu[2], Subhashinie Kariyawasam[2], Lisa Kitto[1] and Amy Mayer[1]

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Alternative methods for the immediate on-site holding of poultry carcasses following catastrophic events are needed. Large laying hen complexes are especially challenged to manage large masses of carcasses in a biosecure and environmentally sensitive manner. Therefore a study was initiated to evaluate the ensiling of Leghorn hen carcasses as a means of virus destruction and biosecure carcass preservation. Sixty five week old fowl (5,460) from a commercial flock were euthanized with CO_2 gas and ensiled in a 2.4m Ag Bag 6.1m long. Two carbohydrate treatments, 14% laying hen mash (HM), and a mixture of 9% hen mash and 5% sugar (HM+S) were mixed and ensiled with the carcasses as they do not contain sufficient sugars for the anaerobic ensiling process. The first 2,880 carcasses were ensiled with the HM (14% by wt) in the first 3m of the bag, and the second group of 2,880 carcasses were ensiled with HM+S in the second half of the Ag Bag. Eighteen carcasses were inoculated with AviPro SOHOL, live virus Newcastle-Bronchitis lyophilized vaccine powder (10,000 doses) reconstituted in distilled water then suspended in equal parts BHI broth (total 40ml). The inoculant contained 250 doses per ml and 1ml was injected into the trachea, and 1ml in the cloaca of each bird. Three bundles of three inoculated birds were bagged in poly-net bags and placed in each of the HM and HM+S treatment sides of the Ag Bag among the other ensiled carcasses. At 3, 7, 14 and 29 days the HM and HM+S treatments were probed (n=6) for pH, and microbial samples. Initial pH on day 3 was not different between the treatments (6.32), however at 7 and 14 days HM+S was significantly lower at 5.41 vs. 6.21 and 5.48 vs. 6.24 respectively. At 29 days the bag was opened and the 6 bags of carcasses were sampled for virus isolation. The ensiling process was both technically and logistically feasible and virus isolation results are forthcoming.

An Integrated Microfluidic Device for Rapid Avian Influenza Virus Capture and Detection

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Rapid detection of avian influenza virus (AIV) is highly desirable during outbreaks or routine AIV surveillance. . In this project, we report our preliminary results in developing a handheld (0.5cm×2cm) disposable device that is capable of rapidly detecting AIV from poultry swabs. This microfabricated device captures the virus particles from a swab sample by employing physical size-based exclusion and identifies the isolated virus using on-chip indirect fluorescent antibody (IFA) staining. The device consists of patterned carbon nanotubes (CNTs) arrays and a microfluidics channel made of polydimethylsiloxane (PDMS), which is an FDA approved biocompatible polymer. The aligned carbon nanotubes are synthesized by aerosol based chemical vapor deposition (CVD) on a micro-patterned ion catalyst thin film to form an enclosed microfluidic chamber of a droplet shape. The top PDMS cover is fabricated with SU-8 mold, and then punctured for fluidic access ports. Particles with diameter similar to CNT gap pore size are trapped inside the forest. CNTs are selectively synthesized on transparent silica substrate with a chemical vapor deposition. The CNTs form a porous structure array with geometries of approximately 50μm in height and 30nm in diameter, as measured by scanning electron microscopy (SEM). The CNT porous structures perform label-free virus trapping and serve as a virus carrier substrate for a subsequent IFA test. First, we characterized size-based capture performance by measuring the capture efficiency using fluorescence labeled particles with 25nm, 100nm and 400nm in diameter. Second, we constructed experimental swab samples by spiking H5N2 virus into a chicken tracheal swab collected from a healthy (virus-free) chicken. By applying on-chip IFA test using AIV H5 monoclonal antibody, strong fluorescent signals were detected at the CNTs porous structures which indicated the H5N2 AIV was trapped and immobilized inside CNTs structure. Furthermore, the carbon nanotube filtration device with captured virus can be integrated with standard AIV detection approaches of immuno- and molecular-assays. Our preliminary results are promising toward for the development of a portable on-chip reaction device for the rapid AIV detection from poultry samples.

Whole Genome Alignment Based One-step Real-time RT-PCR for Universal Detection of All Serotypes and Genotypes of Avian Orthoreoviruses

Yi Tang, Huaguang Lu

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Newly emerging avian orthoreovirus (ARV) variants have been causing major disease problems in broilers, layers and turkeys in Pennsylvania since 2011 to the present time. ARV-affected birds suffered severe viral arthritis or tenosynovitis, enteritis, runting-stunting syndrome and malabsorption syndromes ARV isolation in LMH cell cultures are routinely conducted for all diagnostic cases in laboratory, and we have obtained over 350 ARV isolations from Jan 2011 to July 2015. All ARV isolates are first identified by giant, or "bloom-like" cytopathic effects (CPEs), that are characteristic of ARV infections in LMH cell cultures, and subsequently stained positive for ARV by the fluorescent antibody (FA) test using a fluorescent ARV antibody. In this study, we report our recent research studies in developing a novel one-step real-time RT-PCR (rRT-PCR) for the rapid and universal detection of all ARV strains from clinical specimens. The primers and probe was designed from conserved region of the 5' end of the M1 genome segment based on the whole-genome alignment of various ARV strains including large number of the PA newly emerging field variants or novel strains. The sensitivity of the new rRT-PCR for ARV was determined by using 10-fold serial dilutions of vitro-transcribed viral RNA and 5 titrated ARV strains. Excellent low detection limits were obtained by detecting 10 copies/reactions of viral RNA and from $10^{0.50}$ tissue culture infectious dose ($TCID_{50}$)/100 µL to $10^{0.88}$ $TCID_{50}$/100 µL of virus. The developed rRT-PCR for ARV successfully detected 62 PA ARV field strains tested and reference strains which belonged to different σC genotyping clusters. This new one-step rRT-PCR for ARV had no cross-reaction with other avian viruses. Reproducibility of the assay was confirmed by intra- and inter-assay tests with variability from 0.12% to 2.19%. The comparison of the rRT-PCR results with those obtained from the analysis of 70 clinical samples by virus isolation showed a high level of agreement. Our research findings indicate that this assay is specific and sensitive for the detection of ARV from clinical poultry specimens.

Next-Generation Sequencing (NGS) for Full Genome Sequencing of Newly Emerging Avian Reovirus Field Strains and Other Viruses

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Next-generation sequencing (NGS) methodologies have revolutionized the field of genomics and the field of virus discovery. In clinical virology, NGS is highly efficient, producing an enormous amount of information at low cost in a relatively short period of time. NGS sequencers have enabled significant contributions to multiples areas in virology, including virus discovery, metagenomics (viromes), molecular epidemiology and pathogenesis. Coupled with bioinformatics tools, NGS provides a powerful tool for the identification and characterization of novel viruses. The applications of the NGS methodologies in avian diagnostic virology in our laboratory have yielded full genomic sequences of the PA newly emerging field variants or novel strains of avian reovirus (ARV), a duck H11N9 avian influenza virus (AIV), chicken H5N2 AIV and H7N2 AIV, and turkey burnavirus. These full genomic sequences enable us to better understand how these variant or novel strains structured revolutionary genomic changes, and to select the best candidates of vaccine strains to predict future outbreaks.

DNA from Dust: the First Field-Isolated Genomes of MDV-1, from Virions in Poultry Dust and Chicken Feather Follicles

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Marek's disease (MD) is a lymphoproliferative disease of chickens caused by airborne gallid herpesvirus type 2 (GaHV-2, aka MDV-1). Mature virions are formed in the feather follicle epithelium cells of infected chickens, from which the virus is shed as fine particles of skin and feather debris, or poultry dust. Poultry dust is the major source of virus transmission between birds. Despite both clinical and laboratory data that show increased virulence in field isolates of MDV-1 over the last 40 years, only a few genes have been associated with MDV-1 pathogenicity. At present our understanding of genetic variation in the MDV-1 genome comes exclusively from laboratory-grown isolates. MDV-1 isolates tend to lose virulence with increasing passage number *in vitro,* raising concerns about their ability to accurately reflect virus in the field. The ability to rapidly and directly sequence field isolates of MDV-1 is critical to understanding the genetic basis of rising virulence in circulating wild strains. Here, we present the first complete genomes of uncultured, field-isolated MDV-1, using poultry dust and chicken feather follicle as the source of DNA. We have developed a novel procedure to extract viral DNA from poultry dust and chicken feather follicles, which reduces host and environmental contamination. This DNA was sequenced using the latest Illumina MiSeq high-throughput approaches. We applied new bioinformatics workflows to *de novo* assemble a complete MDV-1 genome, and to analyze the taxonomic diversity found in poultry dust. Future applications of this approach include advancing our understanding of how MDV-1 adapts and attenuates *in vitro,* and providing genome-wide analysis of the evolution of virulence over time in the field.

Survey of Marek's Disease Virus Concentration across Poultry Farms in Central Pennsylvania, USA

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Marek's disease virus, a herpesvirus that is the causative agent of Marek's disease, is a worldwide economic burden on poultry farming. However, little is known about the prevalence and dynamics of this virus in the field. Commercial poultry farming is highly structured, and so important questions are, how does virus prevalence differ across this structure, and how does virus prevalence change within a single level of this structure over time? To answer these questions, we surveyed virus prevalence across farms in Pennsylvania for three years. This involved collecting dust samples from chicken houses and using a qPCR (quantitative polymerase chain reaction) assay specific for non-vaccine Marek's disease virus to assess the virus's concentration in the dust. In total, 4448 samples were collected across 15 operations, and 106 farms. These data were analyzed with generalized linear mixed effects models fit using Bayesian methods. We found that virus prevalence varied considerably in our data. Substantial amounts of variation were attributable to between operation, between grower, and between flock effects. A smaller magnitude of variation was attributable to between house effects and between sample effects. We also identified significant effects of cohort age and seasonality. We were unable to identify a significant effect of production type, but it is unclear whether this was due to a lack of biological importance or due to sampling constraints. Examining the data from within farms over time confirmed many of the patterns discovered by the mixed effect modeling. These data also revealed apparent patterns of virus disappearance and reemergence, where the virus concentration dropped to below detectable levels only to reappear in later samples. Whether this was due to repeated extinction and reintroduction events, or due to virus population dynamics that occurred below the qPCR limit of detection is still an open question.

NECAD Business Meeting

Wednesday
September 16, 2015

Minutes of the 86th NECAD Business Meeting

September 24, 2014

State College, Pennsylvania

- **Call to Order:** The meeting was called to order by Evans, NECAD Chair / Secretary, at 5:35 pm. Members present: 8.

- **Awards:** Mr. Jason Regalado (Virginia Tech) was presented with a certificate and check ($100) for "Best Graduate Student Presentation" – Paper entitled: Evaluation of an Isothermal DNA Amplification Assay for *Salmonella* with Applications for Food Safety.

- **Approval of the Minutes of the 86th NECAD**: A motion was made (Parcells) and seconded (Dunn) to accept the minutes as written. There was no discussion. The minutes were unanimously approved.

- **Report of Financial Statement for the 86st NECAD**: The statement was read by Evans. A motion was made (Dunn) and seconded (Wallner-Pendleton) to accept the statement as written. There was no discussion. The statement was unanimously approved.

- **Old Business**: Don Ritter was approached (Parcells) about the possibility of holding the 87th NECAD in conjunction with the Poultry Health and Processing Meeting (aka "Condems"). However, Ritter indicated that combining meetings would be difficult.

- **New Business**: This was the first year, since partnering with PSSC, that the NECAD Scientific Session (afternoon) was held on the same day as the Symposium (morning). Several members (Dunn and Wallner-Pendleton), including students (Lighty and Regalado), voiced their approval that the single day NECAD meeting schedule should be continued for the 87th NECAD. Evans indicated that there was an increase in the number of submissions for the Scientific Session from industry personnel and raised the question about limiting the number of accepted titles. The membership indicated their approval, but also requested that students be given priority (Dunn, Evans, and Lighty). Evans made the suggestion that the NECAD Chair/Secretary position should be a three year term. Dunn and Wallner-Pendleton indicated that the 86th NECAD was a success and that three years of service would be appreciated.

  The 87th NECAD / PSSC will be held in State College, PA.

- **Adjournment:** A motion was made (Parcells) and seconded (Dunn) to adjourn the meeting. There was no additional discussion. The motion carried unanimously and the meeting was adjourned at 5:50 pm.

Minutes recorded and submitted by:

Nicholas P. Evans, PhD
Chair / Secretary, 86th NECAD

NECAD Financial Statement

September 16, 2015

State College, PA

Beginning Balance - VT Foundation Account 035-8-74119	$2,962.66
Credits	
AAAP Support	3,000.00
Debits	
Award, Best Graduate Student Presentation ($100 personal check written by Pierson as direct donation)	(0.00)
Publication costs for Proceedings 86th NECAD (Treasurer of VT)	(1,250.70)
Speaker Expenses (Treasurer of VT)	(4,000.00)
Cash Gift Assessment (7%) AAAP (VT Foundation)	(210.00)
Ending Balance as of September 1, 2015	**$501.96**
Debits not charged to 035-8-74119	
Speaker Expenses (Pierson-Various Account)	$711.31

Respectfully Submitted,

Nicholas P. Evans, PhD
Chair and Secretary, 87th NECAD