PRD-CAP

Poultry Respiratory Disease Coordinated Agricultural Project

# 2nd Annual PRD-CAP Meeting

**October 19th, 2016**

**Sheraton Greensboro Hotel**
**Greensboro, North Carolina**

USDA

United States Department of Agriculture
National Institute of Food and Agriculture

**AGENDA**

**PRD (Poultry Respiratory Disease)-CAP & NC1180 Joint Meeting**

**October 19-20, 2016**

**Sheraton Greensboro Hotel, Greensboro, NC**

## Wednesday, October 19, 2016 (Room: Colony B)

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| **7:30 – 8:00 am** | **Coffee & Continental Breakfast** |
| **8:00 – 8:15** | ***Welcome & Introduction*** |
| **8:15 – 8:45** | **Perspective on NIFA program –** Dr. Gary Sherman |

**Session I.** Understanding the Ecology of Poultry Respiratory Diseases

***Moderator: Chang-Won Lee***

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| **8:45 – 9:10** | **Mohamed El-Gazzar** | Poultry Networking, Disease Control and Extension Outreach |
| **9:10 – 9:25** | **Patti Miller** | Transmission Dynamics of Respiratory Microorganisms In Backyard Chickens |
| **9:25 – 9:40** | **Timothy Johnson** | Understanding the Respiratory and Intestinal Microbiome of Poultry in Minnesota |
| **9:40 – 9:55** | **John Ngunjiri** | Understanding the Respiratory Microbiome of Poultry In Ohio: Turkeys and Chicken Layers |
| **9:55 – 10:10** | **Calvin Keeler** | Understanding the Respiratory Microbiome of Commercial Broilers in the Delmarva Peninsula |
| **10:10 – 10:30** | **Break** | |

**Session II.** Investigating the Multifactorial Etiology Involving Poultry and Respiratory Diseases

***Moderators: Maricarmen Garcia***

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| **10:30 – 10:45** | **Naola Ferguson-Noel** | Evaluation of the Effect of Infectious Laryngotracheitis CEO Vaccination on Mycoplasma Synoviae in Broiler Chickens |
| **10:45 – 11:00** | **Mary Pantin-Jackwood** | Co-Infection Studies in Chickens and Turkeys with Different Respiratory Pathogens |

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| **11:00 – 11:15** | **Maricarmen Garcia** | Tracking Leukocyte Phenotypic Changes in the CALT, Harderian Gland, Trachea and Spleen of Chickens Inoculated via the Ocular Route with Infectious Laryngotracheitis Virus (ILTV) |
| **11:15 – 11:30** | **Mark Jackwood** | Effects of Air Quality on IBV pathogenesis / Development and Longevity of Protective Immunity Elicited by the Commercial Vaccines and their Effect in Pullets Co-Infected with Other Respiratory Pathogens |
| **11:30 – 11:45** | **David Suarez** | Unification of Molecular Detection Methods for Poultry Respiratory Pathogens |
| **11:45 – 1:00** | **Box lunch** | |

**Session III.** Developing New &Improved Diagnostic Tools, Vaccines, and Novel Preventative Measures

***Moderators: Mazhar Khan***

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| **1:00 – 1:15** | **Mazhar Khan** | Development of Novel Nanoparticle-Base Vaccines for Infectious Bronchitis Virus |
| **1:15 – 1:30** | **Harold Toro** | Infectious Bronchitis Virus S2 Expressed from Recombinant Virus to Confer Protection Across Serotypes in Chicken |
| **1:30 – 1:45** | **Sanjay Reddy** | Use of HVT Vector Vaccines to Protect Against Important Respiratory and Immunosuppressive Diseases of Poultry |
| **1:45 – 2:00** | **Yosra Helmy** | Novel Non-antibiotic Compounds for the Control of Colibacillosis and Mycoplasma |
| **2:00 – 2:20** | **Break** | |

**Session IV.** Education for Prevention and Control of Respiratory Diseases

***Moderators: Eric Benson***

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| **2:20 – 2:35** | **Eric Benson** | Extension Activity Update from University of Delaware and University of Maryland |
| **2:35 – 2:50** | **Michael Hulet** | Extension Activity Update from Penn State University |
| **2:50 – 3:05** | **Rodrigo Gallardo** | Addressing the Needs of Poultry Stakeholders, How to Target Effective Outreach and Applied Research |

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| **3:05 – 3:20** | **Brian Jordan** | Development of an Informational Videos Series Detailing How Biosecurity, House Management, and Farm Operations Affect Respiratory Diseases in Commercial Poultry |
| **3:20 – 3:40** | **Break** | |

**3:40 – 5:00** **Advisor's Comments & Business Meeting**

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## Thursday, October 20, 2016 (Room: Blue Ashe)

**7:30 – 8:00** ***Coffee & Continental Breakfast***

**8:00 – 8:30** ***Welcome & Introduction of NC1180*** **members and guests**

***8:30 – 9:30*** ***NC1180 Business Meeting***

***9:30 – noon*** ***Station Reports on Respiratory Diseases***

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|---|---|---|
| 09:30 – 09:45 | Alabama | Haroldo Toro |
| 09:45 – 10:00 | Connecticut | Mazhar Khan |
| 10:00 - 10:15 | California | Rodrigo Gallardo |
| 10:15 - 10:30 | Delaware | Calvin Keeler |
| 10:30 - 10:45 | Georgia | Mark Jackwood |
| 10:45 - 11:00 | Indiana | Tsang Long Lin |
| 11:00 - 11:15 | Minnesota | Timothy Johnson |
| 11:15 - 11:30 | Ohio | Chang-Won Lee |
| 11:30 - 11:45 | SEPRL-USDA | Mary Pantin-Jackwood |

**11:45** ***Adjourn***

# **Session I.** Understanding the Ecology of Poultry Respiratory Diseases

**Poultry Industry Preparedness and Networking in Ohio**

Mohamed El-Gazzar (PI)

Department of Veterinary Preventative Medicine, The Ohio State University

The purpose of this project is to constitute a platform for the poultry industry in the state of Ohio. This platform is to allow for networking, information sharing and ultimately coordination in disease prevention, control and eradication efforts. Multiple Highly Pathogenic Avian Influenza (HPAI) outbreaks affected the US poultry industry in 2015 and 2016. The spread of the disease in 2015 emphasized the need of this type of platform in facing such scale of disease outbreak. The speed of response including the initial sampling, initial diagnosis can make the difference between controlling the outbreak and allowing it to spread like fire. In case of positive diagnosis, the speed of depopulation and disposal are major factors in the success of outbreak control and disease eradication. Addressing these gaps in Ohio was the focus of our Networking efforts in 2015 and 2016. Our goal was to get the poultry industry including technical service personnel and poultry growers ready to respond quickly in case of an outbreak.

The HPAI outbreaks opened the door for collaboration with multiple organizations towards building this network. In collaboration with Ohio Poultry Emergency Disease Committee (EMD) and the Ohio Poultry Association multiple meeting with the poultry industry have been organized. The message in these meetings, in addition to biosecurity message, is be prepared for the response starting from taking initial samples, to depopulation and disposal. It is ultimately the poultry producer's responsibility to prevent the disease from getting into their operation, but also to control the disease spread in case they have a positive case.
In this spirit, multiple training sessions have been held with individual companies to train them on sampling techniques. Additionally two short videos have been produced in collaboration with Ohio Department of Agriculture to make sampling training available to the as many individuals as possible (https://www.youtube.com/watch?v=B77_ZvLnnxk&feature=youtu.be https://www.youtube.com/watch?v=ubUBLR2B7oU&feature=youtu.be). Depopulation and disposal methods have been discussed and producers are encouraged to prepare for their depopulation and disposal method of choice, each according to their situation.
Building a poultry disease network in the state of Ohio, independent of regulatory bodies is still our goal for this project. Due to the HPAI outbreaks, merge of efforts was needed for better preparedness and crises management. However, in following years, the focus will be to build an independent body which its primary duties will focus on poultry disease epidemiology including data sharing, and coordination on disease prevention, control and eradication.

**Transmission Dynamics of Respiratory Microorganisms in Backyard Chickens**

Patti Miller[1] (PI), Claudio L. Afonso[1], Andrea L. Ayala[2] and Sonia M. Hernandez[2]

[1]Southeast Poultry Research Lab, USDA-ARS; [2]Department of Wildlife Disease and Wildlife, University of Georgia

The agricultural wildlife interface is now associated with rising incidences of agriculturally significant pathogens. The recent rise in unregulated backyard poultry possess risks in terms of biosecurity associated with the proximity of wild birds to poultry. Avian pathogens from backyard poultry and adjacent free-ranging birds have potential for bi-directional pathogen spillovers; among those Mycoplasma gallisepticum, avian pox viruses, and low and highly pathogenic (HPAI) avian influenza. Events, such as the recent HPAI (H5N9) outbreak in the United States, have the potential to decimate the agricultural economy through mass depopulation, quarantines, and trade embargos. While separating wild animals and domestic animals is always the goal of good biosecurity, their interaction is commonplace in non-commercial settings. Here we will assess the role of the factors involved in the risk of transmission of live agents between wild birds and backyard poultry. We will study highly abundant, peridomestic, wild, native bird species in order to establish the importance of factors such as access to food, water, and direct contact to each other for the transmission of respiratory microorganisms. We will administer a commercial live LaSota Newcastle disease virus vaccine to chickens as directed by the manufacturer as a model agent and use a recently developed backyard poultry-wild bird interface system to model the process of respiratory virus transmission in a field setting. We will determine the extent of bidirectional microorganism transfer utilizing Next Generation Sequencing (NGS) of nucleic acids extracted from oral and cloacal swab samples. We will quantitatively measure specific species interactions with backyard poultry and their feed and water sources using radio frequency identification readers, while also measuring the amount of virus shed from the chickens and transmitted each wild bird species. Besides identifying the presence and amount of LaSota in the chickens and passerines, the NGS may also allow for the identification of other microorganisms not present at the onset of the experiment, but present in both the wild birds and chickens after they have interacted. We expect to determine if gregarious, ground foraging species such as sparrows (Emberizidae family) exhibit a proportionately increased level of contact with both chickens, feed and water stations over other species. We hypothesize that the foraging patterns of sparrows may translate to increased contact with chickens and may influence their exposure to avian and environmental sources of virus.

**Understanding the Respiratory and Intestinal Microbiome of Poultry in Minnesota**

Timothy J. Johnson (PI)

Department of Veterinary and Biomedical Sciences, University of Minnesota

The purpose of this project is to define the respiratory bacterial communities present in commercial poultry raised in Minnesota. To that end, we have temporally followed four different antibiotic-free commercial broiler flocks, weekly, for two successive growout cycles. Samples were taken from barn litter and cecum, ileum, and trachea from ten birds at each flock, time point, and growout cycle. Bacterial community profiling was performed using the V4 region of the 16S rRNA, and subsequently assessed using QIIME. We identified distinct bacterial communities for each sample type assessed; however, interesting overlaps emerged. Specifically, many of the dominant operational taxonomic units (OTUs) identified in tracheal samples were classified as *Lactobacillus* species, with the same OTUs identified as dominant taxa in ileum samples. The tracheal bacterial communities were most similar in composition to litter samples, supporting the concept that colonizing bacteria in the trachea result in part from inhaled dust in the barn environment. When comparing commercial chicken and turkey samples, they were highly similar in successive pattern to one another across all tissues, yet distinct based on small differences in taxa. Overall, the work performed thus far provides a detailed glimpse into the true breadth and depth of bacterial species inhabiting the poultry respiratory tract.

**Understanding the Respiratory Microbiome of Poultry in Ohio: Turkeys and Chicken Layers**

John M. Ngunjiri[1], Timothy J. Johnson[2], Chang-Won Lee[1] (PI)

[1]Food Animal Health Research Program, The Ohio State University; [2]Department of Veterinary And Biomedical Sciences, University of Minnesota

The goal of this project is to define bacterial and viral communities in the respiratory tracts of commercial poultry raised in Ohio. Intestinal communities are also defined to allow for a side-by-side comparison with the respiratory communities. Two commercial flocks of turkeys and chicken layers were followed from hatch up to 25 weeks of age. Four to ten birds were sampled at 1, 3, 5, 8, 12 and 16 weeks for both species. The layers were also sampled at 25 weeks. A total of 863 samples were collected including: sinus wash, trachea wash, ileum and cecum. Bacterial community profiling was conducted on the turkey samples using the V4 region of the 16S rRNA gene and subsequent assessment using QIIME and R. A clear distinction was observed between the profiles of respiratory and intestinal bacterial communities with some overlap between sinus and trachea, on one hand, and cecum and ileum on the other hand. Yet, there was a clear difference between sinus and trachea possibly due to sampling or environmental effect. In agreement with the published studies, the intestinal communities were characterized by high levels of bacteroidia in cecum, clostridia in cecum and ileum, and bacilli in ileum. For the respiratory system, bacilli and actinobacteria were commonly found in the sinus and trachea at different levels of abundance. Processing of the chicken samples for sequencing is underway and comparison with turkey data will be made in the near future. Enrichment for virus, viral nucleic acid extraction, and consideration for virome bioinformatics pipeline are also underway. The specific-pathogen flocks raised at the Ohio Agricultural Research and Development Center will be studied to provide data for comparison with the commercial flocks.

**Characterizing the Respiratory Microbiome of Commercial Broilers In the Delmarva Peninsula**

Calvin L. Keeler, Jr. (PI) and Jack Gelb, Jr.

Department of Animal and Food Sciences, University of Delaware

The purpose of this project is to define the viral communities residing in the respiratory tract of commercial poultry raised on the Delmarva peninsula. The first phase of this project entails developing the experimental tools needed to recover viruses from clinical material, extract and sequence viral nucleic acids, and to develop the bioinformatic tools needed to analyze the sequence data. We have initially used clinical submissions from the broiler industry to the University of Delaware Poultry Diagnostic Laboratory as sources of experimental material. A first generation protocol has successfully been used to determine the bacterial and DNA virus composition from tracheal swabs. Bacterial community profiling was performed using the V4 region of the 16S rRNA gene, and was subsequently assessed using QIIME. Virus community profiling was performed by Illumina sequencing of DNASeq libraries, followed by BWA alignment to a database of representative avian DNA virus genomes. Submissions confirmed by the diagnostic laboratory as being infected with infectious laryngotracheits virus have been confirmed by sequencing. In addition, the presence of Marek's disease virus and adenovirus have been identified in these samples. One respiratory sample of uncharacterized cause was found to have *Avibacterium* species as the dominant operational taxonomic unit (OTU). Consequently, this undetermined respiratory clinical submission could be a case of infectious coryza. We are currently evaluating methods to enrich for viral particles of the respiratory tract and to extract nucleic acids (RNA and DNA). An avian virus bioinformatics pipeline is also being developed.

# **Session II.** Investigating the Multifactorial Etiology Involving Poultry and Respiratory Diseases

**Evaluation of the Effect of Infectious Laryngotracheitis CEO Vaccination on Mycoplasma Synoviae in Broiler Chickens**

Naola Ferguson-Noel (PI), Victoria Drouet, Maricarmen García, C. Stephen Roney

Poultry Diagnostic & Research Center, Department of Population Health,
University of Georgia

Objective: To investigate the clinical impact of ILTV CEO eye drop vaccination in MS positive broilers.

Mycoplasma synoviae (MS), and infectious laryngotracheitis virus (ILTV) can cause tremendous economic losses due to mortality, condemnations, cost of treatment and vaccination, and cost of control and monitoring to the commercial broiler industry. Both pathogens are present; and at times, present together in the commercial poultry industry. Four treatment groups consisting of broilers acquired from a commercial source were included in this trial. Groups 2 and 3 were inoculated with MS (strain K6677) at 7 days of age; at 14 days of age, the birds in Groups 3 and 4 were vaccinated via eye drop with commercial CEO ILTV. The birds in Group 1 did not receive any experimental pathogens and served as negative controls. Clinical signs were scored at 5 and 7 days post vaccination. Birds were euthanized and evaluated at 28 and 35 days of age. Birds in the group inoculated with both MS and ILTV (Group 3) experienced the highest mortality (33%) and had the highest airsacculitis incidence, with 80% of the birds affected at both 21 and 28 days post MS inoculation. Based on the clinical sign score assessment, the clinical signs were most severe in the MS and ILTV group (Group 3) due to more severe respiratory signs and apathy, although the scores for conjunctivitis in this group were lower than for the group that receive the ILTV vaccine only (Group 4).

Impact/Summary: These results provide inform the poultry industry as to the increased risk of severe vaccine reactions and exacerbation of respiratory disesase (including airsacculitis) when MS postive broilers are vacccinated with live attenutated ILTV vaccines.

Presentations/Publications:

Evaluation of Infectious Laryngotracheitis CEO Vaccine in Mycoplasma synoviae Positive Broilers. Victoria Drouet Pratt, Naola Ferguson-Noel, Maricarmen García, C. Stephen Roney, Marianne Dos Santos, Ruth Wooten, Tyler Gamble, and D.G. Sandu. American Veterinary Medical Association (AVMA) Annual Convention, San Antonio, TX Aug 5-9, 2016.

**Co-Infection Studies in Chickens and Turkeys with Different Respiratory Pathogens**

Mary J. Pantin-Jackwood (PI)

Southeast Poultry Research Lab, USDA-ARS

Objective: To investigate the multifactorial etiology involving poultry respiratory diseases. We conducted a second co-infection study in SPF chickens with H5N2 and H5N9 LPAIV and IBV to corroborate the results of the first study. Chickens were inoculated with a live IBV Ark strain (virulent field strain) and with a H9N2 or H5N2 LPAIV, by simultaneous or sequential inoculation. Although minimal clinical signs were observed in all groups, significant differences in virus shedding was observed between co-infected groups and groups only challenged with one virus. Groups that received IBV 3 days before challenge with LPAIV shed significantly higher LPAIV titers than groups only challenged with LPAIV. However, when IBV and LPAIV were given simultaneously, lower LPAIV titers were observed. Grossly, no lesions were observed in the trachea, but microscopically typical lesions of IBV infection were present in all birds infected with IBV. AIV virus staining was more frequent in birds challenged with IBV 3 days before challenge with LPAIV, indicating that previous damage to the trachea increases LPAIV replication. In this study, similar to the previous one, no bronchial casts were observed corroborating other pathogens are necessary to reproduce the bronchial casts reported in the field. A manuscript with the results of this study is in preparation.

We will conduct a follow up study in which we will co-infect chickens with LPAIV, IBV and Mycoplasma synoviae (MS). A co-infection study will also be done in turkeys using different respiratory pathogens including LPAIV, NDV, and Mycoplasma meleagridis (MG).

Challenges & potential or actual solutions: For the co-infection studies we have had problems getting IBC and IACUC approvals for using Mycoplasma sp. in our studies. The approvals have finally been obtained so the experiments will be conducted in the next months.

Impacts to date:
The co-infection study provides information on the interaction of two important respiratory viruses affecting chickens: LPAIV and IBV. This information is needed for understanding and controlling respiratory syndromes affecting poultry.

**Tracking Leukocyte Phenotypic Changes in the CALT, Harderian Gland, Trachea and Spleen of Chickens Inoculated via the Ocular Route with Infectious Laryngotracheitis Virus (ILTV)**

M. Krunkosky[1], L.G. Beltran Garza[1], R. M. Gogal Jr.[2], D.J. Hurley[3], M. García[1] (PI)

[1]Poultry Diagnostic Research Center; [2]Department of Biosciences and Diagnostic Imaging; [3]Food Animal Health and Management, Department of Population Health, University of Georgia

Understanding how the avian host responds to ILTV locally and systemically can aid in the development of new vaccines and treatment strategies. The conjunctiva-associated lymphoid tissue (CALT) and the Harderian gland (HG) are important lymphoid tissues involved in local immunity against avian respiratory infections. Together with the conjunctiva epithelia, the trachea epithelia is the second main site of ILTV replication and fluctuation of leukocyte populations during infection or vaccination has not been studied. The spleen is the largest secondary lymphoid organ in chickens and commonly serves as a window for evaluating systemic immunity. Briefly, specific pathogen free (SPF) chickens were mock inoculated with cell culture media or virulent 63140 ILTV strain via the ocular route. At 1, 3, and 5 days post-infection (pi) tissues were collected and leukocytes were isolated from CALT, HG, trachea and spleen. Leukocyte populations and expression of cell surface markers were analyzed by flow-cytometry using commercially available monoclonal antibodies.

The most drastic cell population changes occurred in the CALT of infected chickens, a decrease in leukocytes recovery was observed at days 1 and 3 pi, decrease in MHCII $^{low}$, decrease in CD4+ cells, an increase in MHCII $^{high}$, an increase in IgM positive cells was observed in the CALT at day 3 pi. While MHCI and CD8+ percentage positive cells in CALT of infected chickens remained the same as in mock-inoculated chickens. The most significant changes in the HG were an increase in leukocyte recovery at day 5 pi and an increase in IgM positive cells at days 3 and 5 pi. No changes in the leukocyte recovery rate or percentages of MHCI, MHCII$^{low}$, MHCII$^{high}$, CD4+, CD8+, and IgM cells were detected in trachea or splenic leukocytes from infected chickens. Collectively, the most relevant finding of this study is that during early ILTV infection, when the virus enters via the ocular route, a decrease in CD4+ positive cells in the CALT and no changes in trachea indicates that ILTV infection distinctly modulates the distribution of lymphocytes to favor viral replication. This is the first report of this nature for ILTV and lays the ground to study leukocyte phenotypic changes to CEO vaccination and other respiratory diseases as Infectious bronchitis and avian Mycoplasmas.

**Development and Longevity of Protective Immunity Elicited by the Combinations of Live Attenuated NDV, IBV, ILTV Vaccines in Pullets and the Effect of these Vaccines in Pullets Co-Infected with Other Respiratory Pathogens**

Mark W. Jackwood [1] (PI), Mary Pantin-Jackwood[2], Naola Ferguson-Noel[1], Maricarmen Garcia[1]

[1]Poultry Diagnostic & Research Center, Department of Population Health, University of Georgia; [2]Southeast Poultry Research Lab, USDA-ARS

Objective: To investigate the multifactorial etiology involving poultry respiratory diseases.

Activity 2.2: Study potential interactions between the vaccines used against respiratory disease. Project 2.2.1.

The central hypothesis of this work is that vaccination schedule can affect development and longevity of immunity when multiple live attenuated vaccines are given either simultaneously or sequentially to pullets, and that co-infections with LPAIV, IBV variants, NDV, ILTV or mycoplasma at the time of vaccination can compromise protection.

Progress to date: This study was started on May 21, 2016. Birds were vaccinated at various times with IBV, NDV and ILTV. The first challenge was conducted and birds were necropsied on September 13 to 15, 2016. We are currently processing the samples from those collection days. The next challenge is scheduled for the week of October 3rd (week 24) and necropsy will be the following week. There are 3 additional challenge/necropsy times scheduled for this experiment which will last a total of 36 weeks.

Challenges & potential or actual solutions: Raising birds to maturity is not without its challenges. Aggressive male behavior required us to separate the sexes. Although we lost more birds than anticipated, we still have enough birds for statistically relevant data at each necropsy.

Impacts to date: None

**Evaluate the Effects of Air Quality on the Onset (Infection), Transmission and Severity of Respiratory Disease Caused by IBV as a Model**

Mark W. Jackwood (PI) and Brian J. Jordan

Poultry Diagnostic & Research Center, Department of Population Health,
University of Georgia

Objective: To investigate the multifactorial etiology involving poultry respiratory diseases and specifically examines environmental factors in the pathogenesis and control of respiratory disease.

Activity 2.3: Our objective is to evaluate the effects of reduced air quality on the onset (infection), transmission and severity of respiratory disease caused by IBV as a model system.

Progress to date: We have obtained all of the necessary approvals to conduct this study but have delayed the animal work until the longevity of immunity study is far enough along that animal spaces are available.

Challenges & potential or actual solutions: Part of the challenge with this study is having sufficient animal facilities to conduct the work. The longevity study above needs to take precedence since scheduling it later in the course of the project is not feasible.

Impacts to date: None.

**Unification of Molecular Detection Methods for Poultry Respiratory Pathogens**

David L. Suarez (PI) & Collaborators in diagnostic labs

Southeast Poultry Research Lab, USDA-ARS

This proposal plans a cooperative exercise with select diagnostic laboratories to evaluate, by bioinformatics and laboratory testing, different diagnostic test for respiratory pathogens. The goal of the proposal is to identify the best tests that will be shared with all interested diagnostic laboratories. Because of the highly pathogenic avian influenza outbreak in the United States, a request was made by the Animal and Plant Health Inspection Service (APHIS) to explore alternative H5 subtype influenza test because of issues detecting some isolates. Using the SNP method for identifying primers from highly divergent viruses, several alternative tests were developed that can better identify H5 viruses. Unfortunately these new primer/probe combinations also gave false positives to other influenza hemagglutinin subtypes. On further investigation it was shown that the highly conserved region of the H5 genome in the HA2 region is also highly conserved in other hemagglutinin subtypes. Efforts are ongoing to find primers/probe that are highly conserved for the H5 subtype, but are divergent enough to not also identify other influenza hemagglutinin genes.

Work has also begun on examining the infectious bronchitis RT-PCR test. This includes work on the bioinformatics and testing commonly used assays.

Impacts to date:

Two H5 tests have been identified with broad specificity for all H5 viruses tested, which includes both North American and Eurasian lineages. Both tests are also highly sensitive, and in general are as sensitive as the influenza matrix test. Some cross reactivity is still observed with other influenza subtypes, but with a significant loss in sensitivity. Although not ideal, a clear differential can be observed when comparing the cycle threshold values between the matrix test and the H5 test. If the Ct values are similar, a confirmation of H5 can be made. If the H5 test is 10 Cts higher than the matrix value, then the sample can be determined to be a false positive.

# **Session III.** Developing New and Improved Diagnostic Tools, Vaccines, and Novel Preventative Measures

**Development of Novel Nanoparticle-Base Vaccines for Infectious Bronchitis Virus**

Mazhar Khan[1] (PI) and Peter Burkhard[2]

[1]Department of Pathobiology and Veterinary Science; [2]Department of Molecular And Cell Biology, University of Connecticut

Infectious bronchitis virus (IBV) causes respiratory disease in poultry as well as affecting avian renal and reproductive systems. Controlling of IBV is mainly based on vaccination program. Current available lived attenuated or killed vaccines have been challenged by their effectiveness due to IBV variants and lack of cross-protection.

We have designed novel IBV vaccines by using a highly innovative platform called self-assembled peptide nanoparticle (SAPN). Spike protein comprises major antigenic determinants that induce neutralizing antibodies which makes it a major target of vaccine design. We engineered the coiled-coil sequence of IBV onto the trimeric coiled-coil of several versions SAPNs. DNA oligos coding for the epitope ligated into the plasmid. SAPN constructs were designed and the nucleotide sequence of each SAPN synthesized. Transformation, expression, purification, refolding and biophysical analyses performed. The criteria for the selection of the constructs were based on their aggregation behavior their ability to form nicely shaped and sized nanoparticles as well as their ease of expression in E. coli. Thus, these nanoparticles present are this epitope in a conformation-specific manner to the immune system, hence inducing conformation-specific antibodies with the potential to neutralize the virus in a viral infectivity assay. Four week-old SPF chickens were given 100 µg IBV-SAPN vaccine or refolding buffer intramuscularly. Elicit immune responses in chickens were tested at various time intervals. Each chicken received two boosters two weeks apart. Chickens were bled and sera were separated to assess the immunogenicity. Immunogenicity study is being assessed with assays including ELISA, virus neutralization, lymphocyte proliferation, flow cytometry. SPF Chickens vaccinated with the IBV-SAPN induce higher levels of antibodies than unvaccinated control groups. Studies are progress to evaluate the virus neutralizing effects and cell proliferation responses.

**IBV S2 Expressed from Recombinant Virus to Confer Protection Across Serotypes in Chickens**

H. Toro[1] (PI), Q. Yu, V.L. van Santen[1], F.W. van Ginkel[1]*, K. Joiner[1]

[1]Department of Pathobiology, Auburn University; [2]Southeast Poultry Research Lab, USDA-ARS

*Dr. Frederik van Ginkel passed away unexpectedly in 2016

**Study 1.** The IBV UK 4/91 strain S2 gene was synthesized with codon optimized for chicken. The IBV S2 gene was cloned into the NDV LaSota (LS) vaccine vector. The full-length cDNA clone of pLS/S2-4/91 was sequenced to confirm its sequence fidelity. The infectious clone of pLS/S2-4/91 was used for rescue of the rLS/S2-4/91 virus by reverse genetics technology. The results of characterization of biological properties of rLS/S2-4/91 are as follows: mean embryo death time (MDT) 144 hs; intracerebral pathogenicity index (ICPI) 0.19; i.e., the pathogenicity of the LaSota strain was not altered by the inserted gene. The recombinant virus was titered in different biological systems achieving $10^{8.5}$ egg infectious dose 50%/ml ($EID_{50}$), hemagglutinating activity $2^{11}$, and $10^{8.3}$ tissue culture infectious dose 50 ($TCID_{50}$) /ml. We assessed protection conferred by rLS/S2-4/91 against challenge with virulent Ark. Vaccinated chickens (Mass-only or Mass followed by rLS) were protected against respiratory signs compared to unvaccinated controls. However, blindly evaluated clinical signs and viral loads both in tears and trachea (determined by qRT-PCR) did not achieve statistical significance. The attenuated IBV 4/91 vaccine, used in a combined prime-boost regime with attenuated Mass, has been shown by others to confer protection against challenge with IBV strains showing reduced S1 sequence similarity with IBV 4/91 (e.g. the phylogenetically distant Chinese IBV QX type). The current results show that its cross-protective capabilities do not reside in the S2 protein.

**Study 2.** We compared protection conferred by the S1 subunit alone with protection of recombinant proteins containing the S1 domain and S2 ectodomain. It is generally accepted that the spike protein S1 subunit is responsible for attachment to susceptible host cells, while S2 mediates viral envelope fusion with the host cell membrane. However, we found that the S2 domain contributes substantially to binding affinity to relevant chicken tissues and is relevant in conferring protection against challenge.

**Study 3.** We tested the stability of kidney-cell-adapted ArkDPI (developed during year 1) during 5 back-passages in embryonated chicken eggs and further evaluated protection against homologous virulent Ark challenge. Viral loads (IBV RNA by qRT-PCR) in allantoic fluids determined 3 days after inoculation in SPF embryonated eggs remained similar through passages indicating absence of positive and negative selection. Sequences for all 5 samples for each of the five egg passages showed stability of the S gene of CEK-adapted ArkDPI vaccine virus during passages in embryonated eggs. Amino acid frequency in NSP and S proteins of kidney-cell adapted ArkDPI-derived vaccine after 5 embryo back-passages assessed by next generation sequencing showed that homogeneity was maintained or increased at all distinct positions of the CEK-adapted virus. Protection conferred by increasing dose of kidney-cell-adapted IBV ArkDPI-derived vaccine (2nd embryo back-pass) administered at 1 day of age showed effective protection against Ark virulent challenge based both on respiratory signs and viral load in lachrymal fluids. Thus, 1) ArkDPI-adaptation to CEK reduces population

heterogeneity and changes in S1 do not revert after five back-passages in embryonated chicken eggs; 2) CEK-adapted ArkDPI-derived vaccine after back passage in embryonated eggs confers protection against virulent Ark challenge; and 3) Ark-DPI vaccine adaptation to CEK improves this type of vaccine as it likely reduces the emergence of vaccine-derived IBV Ark-like strains.

**The Use of Turkey Herpesvirus Vector Vaccines to Protect Against Respiratory Diseases of Poultry**

Sanjay M. Reddy, Owais Khan, Blanca Lupiani (PI)

Department of Veterinary Pathobiology, Texas A&M University

The use of Turkey herpesvirus (HVT) as a viral vector to express immunogenic proteins has been shown to be successful in the poultry industry. The objective of this work was to generate recombinant HVT vectors expressing antigenic/antibody neutralizing proteins from Infectious bronchitis (IBD) and Newcastle disease (ND) viruses. Using a BAC clone of HVT, we generated recombinant HVT viruses expressing S1 gene of IBDV Massachusetts strain (rHVT-IB) or F gene of NDV Lasota strain (rHVT-ND). These genes were cloned into site A of the HVT genome, which was earlier shown to stably express infectious bursal disease VP2 gene without hampering the viral vector growth. S1 and F cDNA clones were generated by RT-PCR from virus genomic RNA. Expression of the F gene in rHVT-ND was detected by immunofluorescence assay (IFA), but not by Western blot analysis using NDV specific polyclonal antibodies. One the other hand, rHVT-IB failed to react with IBV specific polyclonal antibodies both by IFA and Western blot analysis, even though full length gene was detected by PCR analysis. Studies are underway to identify problems associated with IBD virus S1 protein expression. In vivo studies with rHVT-ND and rHVT-IB will determine if the recombinant HVT vectors provide protection against virus challenge.

**Novel Non-Antibiotic Compounds for the Control of Avian Pathogenic *E. Coli* (APEC) and Mycoplasma Infections in Poultry**

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Study 1: Develop novel small molecule inhibitors to enhance the control of APEC in poultry: Colibacillosis is a major worldwide endemic bacterial disease of poultry. Colibacillosis is caused by avian pathogenic *E. coli* (APEC), which affects chickens, turkeys and other avian species. Infections are associated with high morbidity and mortality along with reduction in egg production and meat quality resulting in severe economic losses. Currently, APEC infections are controlled by vaccinations or antibiotic treatments. However, the effects of these treatments are limited. Here, we proposed to identify novel compounds that inhibit APEC quorum sensing (QS), a mechanism that is associated with APEC virulence. Using the bioluminescent indicator *Vibrio harveyi* BB170 (AI-1$^{+}$, AI-2$^{-}$), we screened the culture free supernatant of APEC O78 grown in the presence of a library of ~4,200 compounds (at 200 µM concentration) to identify compounds that inhibited the QS auto-inducer (AI-2) activity. Inhibition of AI-2 activity was determined by measuring the induction of bioluminescence in the indicator bacteria. Screening supernatants of cultures exposed to compounds (4,120) that did not affect the growth, we identified 10 compounds that significantly inhibited the AI-2 activity and showed minimal cytotoxicity on Caco-2 cells (a human intestinal cell line) and no hemolytic activity to sheep red blood cells (RBCs). The compounds also significantly affected the survival of APEC O78, O1 and O2 in chicken (HD-11) and human macrophages (THP-1) and Caco-2 cells. Additionally, most compounds significantly reduced biofilm formation and motility. The expression of several virulence and biofilm associated genes of APEC was also differentially affected by these compounds. Further, these compounds demonstrated good efficacy against APEC infections in *Galleria mellonella* (wax moth *in vivo* model).

In a related study, we also screened the small molecule library (~4,200 compounds) for growth inhibition of APEC O78. Thirty three small molecules completely inhibited APEC O78 growth. Eight of the 33 molecules were cidal and possessed a minimal inhibitory concentration (MIC) that ranged between 12.5 µM and 200 µM. These eight compounds showed a relatively low cytotoxicity in Caco-2 and HD-11 cells, a decreased hemolytic activity against sheep RBCs, and reduction in internalized APEC O78, O1 and O2 strains in Caco-2, HD-11, and THP-1 cells.

These results demonstrate the potential of quorum sensing- or growth inhibitors in controlling APEC. Studies to determine their impact on APEC infection in chickens are in progress.

Study 2: Develop novel small molecule inhibitors to enhance the control of *Mycoplasma* infection in poultry: *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the major causes of mortality in poultry and are associated with a variety of symptoms such as sinusitis, airsacculitis, nervous signs, swollen joints, enlargement of the sternal bursa, and depressed growth. Here, we screened approximately 2000 compounds against MG for growth

inhibition. We identified 360 small molecules that completely inhibited MG growth. Further studies are underway to characterize these hits in secondary assays and identify potential compounds for *in vivo* evaluation in chickens.

# **Session IV.** Education for Prevention and Control of Respiratory Diseases

**Control of Endemic, Emerging and Re-Emerging Poultry Respiratory Diseases in the United States (PRD-CAP)**

Eric Benson[1] (PI), Jonathan Moyle[2,] and Robert Alphin[1]

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Reportable disease outbreaks have a way of changing things. In 2015, the highly pathogenic avian influenza outbreak was described as the worst animal health disaster in US history. The UD – UMD group responded through targeted biosecurity training session in 2015, quickly addressing the Year 1 and Year 2 objectives. In Year 1, the group provided local biosecurity training reaching approximately 615 participants on the Delmarva Peninsula. In Year 2, the group provided training to the National Association of County Agriculture Agents on basic biosecurity and discussed the Delmarva biosecurity training at a national poultry meeting, in both cases, leveraging the local experiences for national audiences. In Year 1 and Year 2, the project team leveraged PRD activity along with separately funded sessions, providing synergistic biosecurity and response training for USDA National Veterinary Stockpile 3-D contractors, USDA Veterinary Services personnel, and for international veterinarians. For Year 3 (2017), the UD – UMD team is using a two pronged training approach in which (a) cohosting poultry respiratory disease training sessions with Delaware Ag Week sessions oriented for regional broiler growers and (b) a one to two day PRD add on training program for an international poultry disease program funded for 2017. This two prong approach would allow high grower participation at the Delaware Ag Week while adding access to an interested and applicable international audience, leveraging program costs.

**Educate Stakeholders for Prevention and Control of Respiratory Diseases**

Michael Hulet[1] (PI), Eva Wallner-Pendleton[2], Patricia Dunn[2], Phillip Clauer[1], And Gregory Martin[3]

[1]Department of Animal Science; [2]Department of Veterinary and Biomedical Sciences; [3]Extension Administration, Pennsylvania State University

Activity 1: Farm Plan Program was developed by Dr. Paul Patterson for flocks dealing with catastrophic disease events. Eleven poultry companies met with Dr. Gregory Martin to develop the plans (Plainville Farms, Joe Jurgielewicz and Sons, Ltd., Kreider Farms, Clark's Feed Mill, Tyson Foods (New Holland, PA), Esbenshade Farms & Mills, JM Hatchery, Freebird Chicken, Perdue Farms (Fredricksburg, PA), and Empire Kosher Poultry). Poultry companies growing turkeys, Pekin ducks, layers, and broilers were represented. Educational material (posters and brochures) were prepared on the following topics: Respiratory Diseases of Small Poultry Flocks, Biosecurity Protecting Your Birds from Disease, Successfully Raising a Small Flock of Laying Chickens, and HPAI Emergency Response Plan (English and Spanish) were presented on various functions. Poster presentations were made at the National Poultry Science Meetings entitled "Educational Program Development in Response to 2014-2015 Avian Influenza Outbreak", and in a symposium given by Dr. Moyle and Martin at the meeting entitled "Assessing How Poultry Growers Respond to Current Biosecurity Demands". Drs. Pendleton and Dunn gave presentations to the PVMA Three Rivers Veterinary Conference on backyard poultry and medicine; PASA Conference (two day) on detecting and preventing disease; FarmFest (two day, Pendleton, Dunn, and Clauer participated); Dr. Martin presented biosecurity lecture at North American Manure Meeting; and Dr. Hulet gave two backyard bird care and management presentations at The Poultry School at Stone Barns Center, in New York. At a recent Pennsylvania Ag Progress meeting Drs. Clauer, Martin and Pendleton gave presentations on "Management Practices for the Care of Backyard Poultry, Movement to Cage Free Production: impact on Consumers, and Avian Influenza and What you Should do to Protect Our Backyard Poultry Flock. Dr. Pendleton also participated in educational meetings for veterinarians and regional and national gamebird meetings (NAGA, PA Gamebird Meeting) giving presentations on "Diagnostic Testing for Poultry Respiratory Diseases" and "Biosecurity for Gamebirds Post HPAI".

Activity 2: Posters were displayed at 57+ county fairs, organic and pastured poultry meetings, Pennsylvania Farm Show, and regional extension meetings. Four brochures were prepared and 17,000+ were distributed. Twelve meetings in multiple locations in Pennsylvania were sponsored by extension and PA Department of Agriculture to discuss respiratory disease throughout the state. Two veterinary practitioners meetings and necropsy labs were conducted on poultry health concerns. Educational workshops were conducted throughout the region targeting on training in biosecurity for small flocks, 4-H, integrated companies, and contract growers. Phillip Clauer sent emails to Extension Offices aimed at 4-Hers to remind them of the HPAI threat and encourage them to participate in events without birds. Several meetings were held for small independent crews and companies to discuss risk factors for disease introduction and disease spread when handling poultry. One international meeting

with 28 participants from Chile, South Africa, India, Canada, and the United States was conducted at the International Expo in Atlanta for training in poultry handling and biosecurity.

Activity 3. Two articles on respiratory disease and biosecurity to prevent the spread of disease were published in The Game Bird Bulletin. Support was given to the PA Game Commission and PA Game Breeders Association with Biosecurity presentations by Dr. Justin Brown (High Path Avian Influenza), Dr. Gregory Martin (Biosecurity: Cleaning and Disinfection), Dr. Eva Pendleton (Necropsy and Tissue Submission), Dr. Paul Patterson (Penn State Farm Plan Template for Developing Flock Plans for Catastrophic Events).

Impacts to date:

Brochures and posters were created to inform Veterinarians, Extension Educators, Gamebird Producers, Small Organic and Pastured Poultry Operations and Backyard, Hobby, and Exhibition Growers and the general public on the importance of controlling respiratory diseases. Over 100,000 contacts were made through distribution of brochures, poster presentations, presentation at local, regional, and national meetings.

Publications

Hulet R. M., E. Wallner-Pendleton, P. Clauer, G. Martin, P. Dunn, and P. Patterson, 2016. Educational Program Development in Response to 2014-2015 Avian Influenza Outbreak. Poultry Sci. 95 (Suppl.1): 326P.

Moyle, J. and G. Martin, 2016. Assessing How Poultry Growers Respond to Current Biosecurity Demands. National Poultry Extension Workshop. New Orleans, LA,July 11 – 14.

Martz, Michael, 2016. Biosecurity Plan for Dealing with Pathogenic Avian Influenza, 2016. Pennsylvania Game Bird Meeting, State College, PA, February 22 – 24.

**Addressing the Needs of Poultry Stakeholders, How to Target Effective Outreach and Applied Research**

Rodrigo A. Gallardo[1] (PI), Rüdiger Hauck, Maurice Pitesky[1], H.L. Shivaprasad[1], Gabriel Senties-Cue[1], Theodore Derksen[1]

[1]Department of Population Health and Reproduction, University of California, Davis;
[2]California Department of Food and Agriculture (CDFA), [3]California Poultry Federation (CPF)

While specific numbers are not available, the number of backyard (BY) poultry submissions to the California Animal Health and Food Safety Laboratory (CAHFS) for diagnosis has increased by 383% over the last 6 years. The lack of appropriate biosecurity measures, inconsistent vaccination, long lifetime span, and poor access to veterinary care are common practices with respect to BY and small flock chickens. The combination of all these factors makes them vulnerable to diseases. Consequently, BY poultry can be both sentinels and amplifiers of several diseases including "foreign diseases" such as avian influenza (AI) and exotic Newcastle disease (END).

Despite the difficulties, identifying small flocks and building accurate databases of poultry holders is of great importance for targeted training and information diffusion. Seroprevalence studies are crucial to assess the exposure of poultry flocks to different pathogens and it is helpful in detecting their potential as reservoirs of disease. This strategy has been used to identify disease risk in BYF. Respiratory pathogens are one of the most common causes of disease in commercial and non-commercial poultry operations. These pathogens not only cause respiratory signs but also reproductive, urogenital and production losses ending, in some cases, in mortality. This limits the potential of the flock and impairs the well-being of the birds. Respiratory pathogen seroprevalence information in small BYF is limited.

Hypothesis:
Our main goal is to locate and educate small flock owners. In addition, social network analysis will be used to understand the different aspects and interactions of small poultry practices in two demographically different populations in California. We hypothesize that using our proposed methodology, an appropriate outreach strategy will be generated allowing for improved outreach, disease prevention and applied research.

Specific objectives:

- Facilitate a one-day intensive lecture and experiential learning program at two different strategically selected locations in the state
- Train disseminators of information in the community, so they can train their contacts in the network (targeted train the trainers strategy)
- Understand, using Social Network Analysis (SNA), the different interactions of small poultry and comparing two SNA overtime to evaluate the effectiveness of the training sessions
- Detect small flock owners by geo-surveys
- Conduct a pilot small flock respiratory disease seroprevalence study

Update on activities:

- Workshops will be targeted to 4H since we have detected in previous work that 4H has a big role in poultry education and this function is not interconnected.

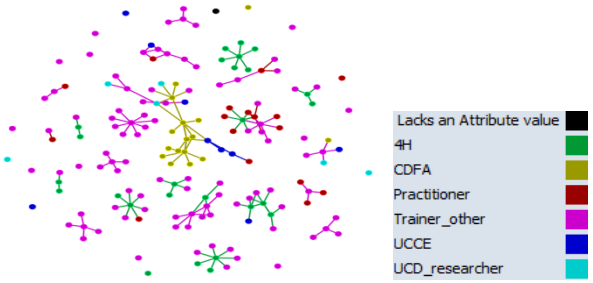


**Figure 1.** Green dots correspond to 4H poultry related people. They tend to share information with others but they are not really connected with extension, universities or the state.

- The first workshop will take place in San Luis Obispo because it has almost 300 adult 4H volunteers and more than 1,400 enrolled youth. We already have a date: November 4th, a flyer and topics to be covered (Figure 2). Hands-on activities will be performed exploring experiential learning as an extension tool.


**Figure 2.** Registration and program flyer. This is being distributed by the 4H program lead at San Luis Obispo (SLO). The workshop will be held at the SLO county offices

- During this meeting a SNA will be performed in order to understand better the dynamics of the poultry network in SLO. After a couple of months we will perform another SNA to see if there was a change in the networking of the poultry participants thanks to the extension strategy.
- We have been constantly working on the Geo-survey in order to identify more and more small flocks across the state; this survey will also be promoted during the workshop.

Pilot Research: Backyard poultry as a reservoir for respiratory diseases

Respiratory diseases are fairly common in small flocks; our states' diagnostic laboratory has estimated that almost 14% of the diagnoses from submitted small poultry flock cases are associated with respiratory disease (Stinson and Mete, 2013). On the other hand respiratory diseases are one of the most common diseases diagnosed in commercial poultry.
Using the Geo survey (Figure 3) database and with the help of poultry extension at UCD, we have sampled more than 500 birds from 40 small flocks located close and far from commercial poultry premises. We arbitrarily set the distance of 4 miles to determine if premises are close or far from commercial poultry. The idea is to determine if backyard flocks pose a menace to commercial poultry production.

We have focused on respiratory diseases of economical importance and we have assessed the exposure of small flocks to these diseases studying their seroprevalence based on antibody titers measured by commercial ELISA's. We covered antibodies against: MG, MS, IBV, NDV, ILT, ORT and AI.

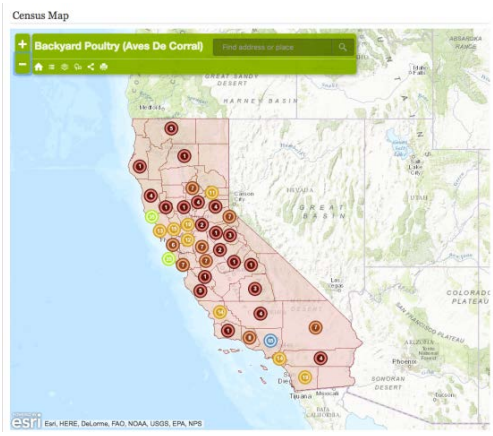


**Figure 3.** Backyard poultry census map from California

We hypothesize that Backyard poultry will show a respiratory disease antibody profile reflecting their environment acting as sentinels of certain diseases

Our Objectives are:

1. Determine biosecurity measures, health and management strategies of BYF
2. Assess the seroprevalence of chicken respiratory diseases in backyard flocks in relation to the proximity to commercial poultry facilities

Results:

- Survey results reflecting 40 flocks:
    - 14/40 (35%) obtain their chickens from feed stores, 16/40 (40%) obtain them from friends, 9/40(23%) from an NPIP hatchery and 1/40 (2.5%) hatch their own chickens.
    - 28/40 (70%) of the flocks surveyed do not use dedicated clothes when working with poultry.
    - When we asked them how often did they clean their coop 2/40 (5%) responded annually, 9/40 (22.5%) responded monthly, 11/40 (27.5%) mentioned semi-annually and 18/40 (45%) responded weekly.
    - 55% of the owners were familiar with our state diagnostic laboratory (CAHFS)
    - Finally 35% of the flock owners used a laboratory or a veterinarian to monitor or investigate health related issues.

- Seroprevalence results reflect 37 flocks (500 birds):
    - Flock size ranged from 2 to 100 birds
    - Far from commercial premises (>4miles): 27 premises, 329 birds
    - Close to commercial premises (<4miles): 10 premises, 137 birds

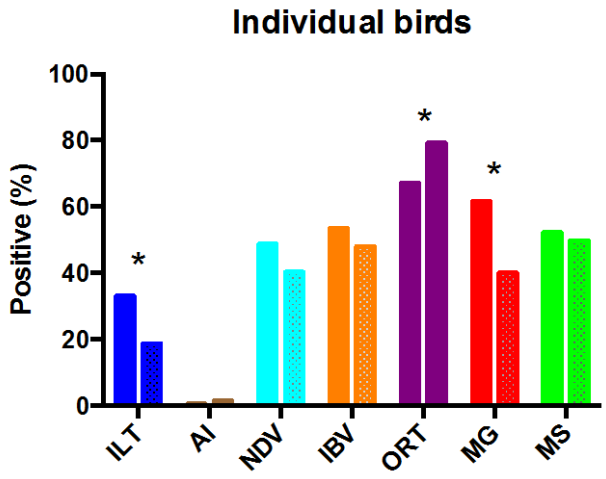


**Figure 4.** Percentage of birds positive to different respiratory pathogens antibody detection. Patterned bar represent birds far from commercial premises. Asterisk indicates significant differences ($p<0.05$)

Interestingly backyard flock birds close to commercial premises have an increased ILT and MG seroprevalence compared with birds far from commercial premises. On the other hand ORT is more seroprevalent in birds far from commercial premises (Figure 4). If we look at the results by premise we see that ILT is considerably higher in premises close to commercial premises, same happens with MS, on the other hand ORT is higher in flocks far from commercial premises (Figure 5).

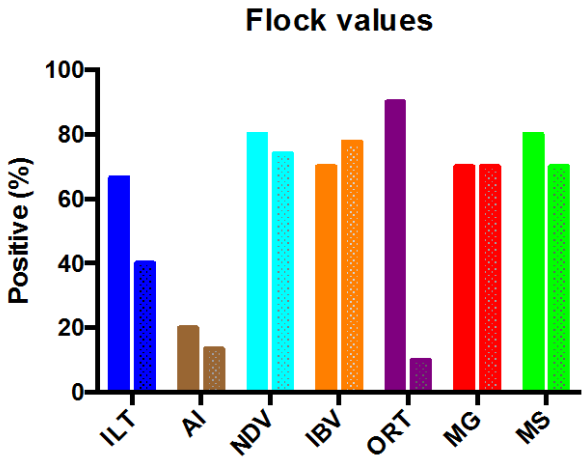


**Figure 5.** Percentage of flocks positive to different respiratory pathogens antibody detection

Presentations:

Derksen T., R.A. Gallardo. Backyard Poultry as a Reservoir for Commercial Poultry Respiratory Diseases. California Poultry Federation Quality Assurance Meeting, Modesto, CA. June, 2016.

**Development of an Informational Video Series Detailing on How Biosecurity, House Management, and Farm Operations Affect Respiratory Diseases in Commercial Poultry**

Brian James Jordan[1] (PI), Brian Fairchild[2], Michael Czarick[2], and Casey Ritz[2]

[1]Poultry Diagnostic & Research Center, Department of Population Health;
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The goal behind this project is to develop digital media content that educates the poultry industry on all the aspects of poultry, not just biosecurity, which can affect the development of respiratory disease in commercial poultry. The approach of this project will be applicable and direct, via a short series of YouTube style videos, which can be distributed worldwide with no cost. Our expected outcome of this project is to set various high quality, informational videos outlining the effects of poultry farm operation on the development of respiratory disease. The videos will cover many topics both directly and indirectly related to respiratory disease, and will provide insight into how operations may be modified or improved to combat these respiratory pathogens. The potential impact of this video series is sizeable. We will now have a digital video media designed for the changing population of the US, allowing us to reach more people than through traditional print media distribution. Furthermore, using a site like YouTube to distribute these videos will also give us data points on how many people are actually watching the videos (number of views). But most importantly, we will be distributing helpful information regarding respiratory disease to the poultry industry in an easily accessible and consumable format.

**Poultry Medicine Workshops and Noncommercial Poultry Outreach**

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As it relates to Highly Pathogenic Avian Influenza (HPAI) preparedness, and in addition to working with the commercial poultry industry, our outreach efforts in the State of Ohio were extended to noncommercial poultry owners. A brochure is produced in collaboration with the Ohio Poultry Emergency Disease Committee (EMD) (https://vet.osu.edu/sites/vet.osu.edu/files/documents/extension/Noncommerical%20Poultry%20Trifold%20%20Final%20Website%20July%202016.pdf). The brochure contains information about the HPAI, the outbreak in 2015 and 2016, but the main message is for poultry owners to report any high mortality. The goal is to increase the chance of detecting any potential outbreak in noncommercial poultry. This brochure has been distributed throughout the state of Ohio using the structure of OSU Extension being represented in each county throughout the state. This brochure have reached all 88 counties of Ohio, it has reached each of 9,000 plus 4-H students with poultry projects. Through the Extension offices the brochure is being distributed to feed stores, agriculture supply stores, poultry shops, shows, swab meets and any place that is significant in the noncommercial poultry activity. Through this distribution system we are hoping to reach as many poultry owners as possible and encourage them to report high mortality. More than 30,000 copies have already been distributed throughout the state.

Another aspect of our Extension as a part of multidisciplinary project is the Poultry Medicine Workshop (https://vet.osu.edu/extension/conferences-and-workshops/poultry-medicine-workshops), offered to non-poultry veterinarians. Targeting small animal and mixed animal veterinarians, three separate workshops will be held in the first week of October, in 3 different Ohio cities. Multi-speaker comprehensive program has been designed to introduce veterinary practitioners that typically work with small animal and mixed animal practice, to poultry medicine. The goal is to provide veterinarians with the necessary knowledge and skills to accept poultry clients. Noncommercial and urban backyard poultry producers are a growing and underserved population that will require more veterinary involvement, especially when the Veterinary Feed Directive (VFD) takes effect in 2017.

We aim to create a front line of veterinarians that can serve the noncommercial poultry. This frontline represents an opportunity to collect poultry disease data from what is typically hard to reach population. Eventually the data from the commercial network and the data from noncommercial poultry could be combined in one comprehensive data base to represent the poultry disease status in the State of Ohio.