IOWA EGG INDUSTRY SYMPOSIUM

EMERGING DISEASES AND CREATIVE SOLUTIONS

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IOWA EGG INDUSTRY SYMPOSIUM AGENDA

EMERGING DISEASES AND CREATIVE SOLUTIONS

Thursday, November 14, 2024

7:30 – 8:15 a.m. .... **Registration**, Garden Room

MORNING SESSION I .... **Moderator:** Yuko Sato

8:15 – 8:30 a.m. .... **Welcome:** Patrick Halbur, *ISU VDPAM Department Chair*

8:30 – 9:45 a.m. .... **Flash Talks – Moderator:** Mohamed El-Gazzar

1. Development and Validation of TaqMan Real-Time Quantitative PCR Assay: An Alternative for Improved Diagnosis of Spotty Liver Disease – *Eman Gadu*
2. Salmonella Vaccine BBS 866 Reduces Cecal Colonization and Systemic Dissemination of DSC Enteritidis in Chickens – *Samuel Whelen*
3. Screening Novel Biocides for Rapid Killing of Salmonella Isolates of Concern – *Maya Encinosa*
4. Efficacy of Novel Compounds with Biocidal Activity against Campylobacter – *Lucas Thompson*
5. Nanopore Sequencing: Real-Time Genomic Solutions for Rapid Diagnostics of Avian Foreign Animal Diseases – *Maria Chaves*
6. The Unseen Challenge: Distinguishing Pathogenic from Non-Pathogenic Avibacterium Paragallinarum in Commercial Layers – *Mostafa M.S. Shelkamy*

9:45 – 10:00 a.m. .... **Coffee Break**

MORNING SESSION II .... **Moderator:** Yuko Sato

10:00 a.m. – 12:00 p.m. **Panel discussion:** What do we know about avian metapneumovirus in the US and how do we get out of this?

Carol Cardona, *University of Minnesota*

Ivan Alvarado, *Merck Animal Health*

Michaela Olson, *Wilson Vet. Co.*

12:00 – 2:00 p.m. .... **Lunch and Tour of Iowa State University Veterinary Diagnostic Laboratory**

AFTERNOON SESSION .... **Moderator:** Kevin Stiles, *North Central Poultry Association*

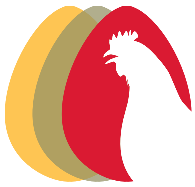
2:00 – 4:00 p.m. **Panel discussion:** What is HPAI in dairy cattle? How can the cattle and poultry industry work together to solve this problem?

Drew Magstadt, *Iowa State University*

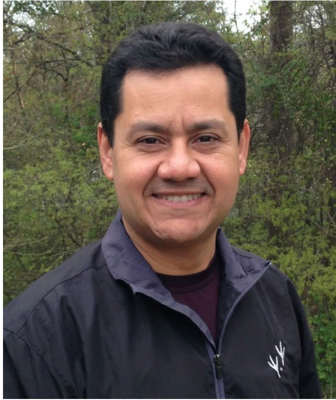
Carrie Cremers, *Hormel Foods*

Nancy Barr, *Michigan Allied Poultry Industries*

Lee Maassen, *Farming Operation and Midwest Dairy Association – IA division*



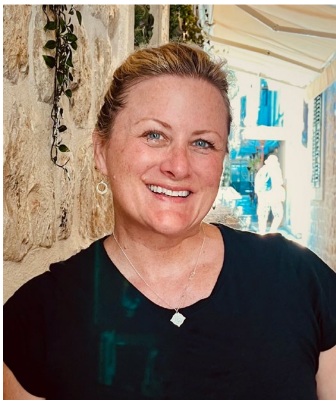
SPEAKERS



DR. IVAN ALVARADO

Merck Animal Health | ivan.alvarado@merck.com

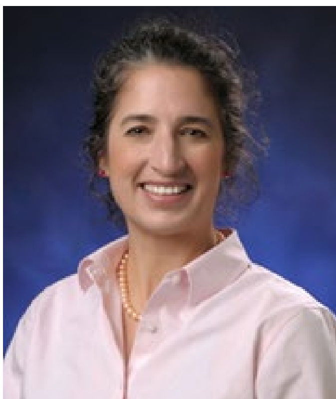
Dr. Alvarado obtained his Veterinary Medicine degree in Colombia and has been practicing poultry medicine for over 20 years, starting as a commercial layer and a broiler breeder Veterinary supervisor in Colombia. After finishing his Master, Doctoral and Post-doctoral studies at the University of Georgia, Dr. Alvarado joined Lohmann Animal Health as a Technical Services Veterinarian in the U.S. In 2012, Dr. Alvarado joined Merck Animal Health as a Global Director of Technical Services and became the Director of the U.S. Poultry Technical Services Team in 2013. During the last years, Dr. Alvarado has been part of the U.S. Strategic Development and Innovation Team. Dr. Alvarado is an active member of AVMA, AAAP and ACPV, currently serving as a member of the AAAP board of directors.



DR. NANCY BARR

Michigan Allied Poultry Industries | nancy@mipoultry.com

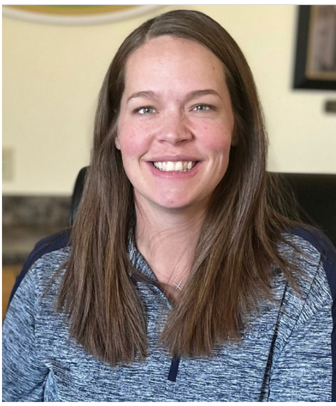
Dr. Nancy Barr is the Executive Director of Michigan Allied Poultry Industries. Prior to leading MAPI, Nancy was the Assistant State Veterinarian for the Michigan Department of Agriculture and Rural Development (MDARD). Nancy spent 23 years in various roles at MDARD and managed the poultry program for more than a decade. As the poultry program manager, she oversaw the H5N1 low path avian influenza surveillance component of the NPIP, managed avian reportable diseases, and developed Michigan's first state avian influenza response and containment plan (ISRCP). Dr. Barr developed and oversaw annual HPAI preparation exercises with the Michigan poultry industry and regulatory partners. Dr. Barr received her Bachelor of Science in Zoology and Doctor of Veterinary Medicine degrees from Michigan State University.



DR. CAROL CARDONA

University of Minnesota | ccardona@umn.edu

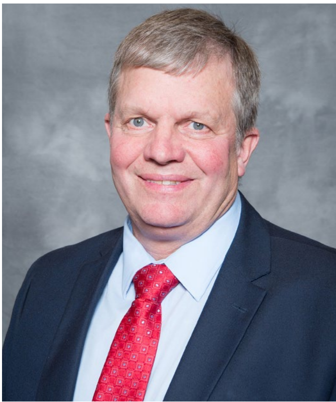
Dr. Carol Cardona is the Pomeroy Chair in Avian Health for the College of Veterinary Medicine. In addition to her expertise in avian influenza, Dr. Cardona is interested in zoonoses (infectious diseases that are transmitted between animals and humans) and the role that poultry play in human health and well-being. She also serves as co-director/co-principal investigator of the Secure Food System at the University.



DR. CARRIE CREMERS

Hormel Foods | cmcremers@j-ots.com

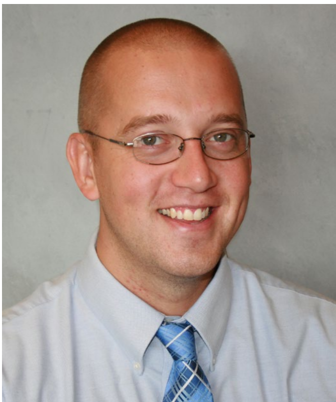
Dr. Cremers graduated from the University of Minnesota and practiced dairy medicine in Minnesota. After dairy practice, Dr. Cremers worked with broiler operations located in Minnesota, Wisconsin and Alabama. During this time, Dr. Cremers graduated from the University of Georgia with her Masters of Avian Health and Medicine and successfully completed her board certification and became a diplomate with American College of Poultry Veterinarian. Currently, Dr. Cremers works for Jennie-O, a subsidiary of Hormel Foods. She is the Manager of Technical Services and Animal Welfare for the company and oversees the technical aspects of breeders, hatcheries, grow out, research and processing plants. Dr. Cremers lives in Minnesota with her husband Andy and two beautiful boys at the ages of 3 and 1. They also own and operate their own dairy farm.



LEE MAASSEN

Farming Operation and Midwest Dairy Board | leeandem@mtcnet.net

We are a multi-generational dairy farm. I farm with my three sons and their families. We milk 1,800 cows, have 27 employees and farm 1,500 acres of land. We also raise all the offspring females and males. The females go into the milking herd and the males are sold as either breeding bulls or as dairy beef. We love making the best nutritious, high-quality milk. Animals are given the most comfortable environment possible; employees are treated with respect and land resources undergo the best stewardship practices possible. We care for our cows, employees, land, water, air in the best way possible. Lee has been involved with the Midwest Dairy Board since 2012 and currently is chairman of the Midwest Dairy Association – IA Division.



DR. DREW MAGSTADT

Iowa State University | magstadt@iastate.edu

Dr. Drew Magstadt is a clinical associate professor and veterinary diagnostician at Iowa State University Veterinary Diagnostic Laboratory. In addition to necropsy floor duties, Dr. Magstadt coordinates bovine molecular testing submissions and BVDV testing. His research interests include various infectious diseases in production animal species.

DR. MICHAELA OLSON

Following her undergraduate in Animal Science at the University of Minnesota, Dr. Olson went on to complete her Doctorate of Veterinary Medicine at the University of Minnesota College of Veterinary Medicine. Upon graduation, she joined Wilson Vet Co., a fully-independent, poultry-focused veterinary practice. Wilson Vet Co. serves over 72 million commercial layers and layer pullets plus a variety of other poultry species across 23 states. When not practicing veterinary medicine, Michaela resides on a few acres near New Richland, Minnesota, and enjoys spending time with her husband, Tyler, their dogs, Goose and Griffin, and tending to her beautiful gardens and houseplants.

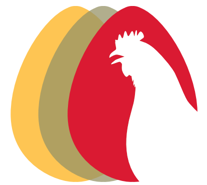
DEVELOPMENT AND VALIDATION OF TAQMAN REAL-TIME QUANTITATIVE PCR ASSAY: AN ALTERNATIVE FOR IMPROVED DIAGNOSIS OF SPOTTY LIVER DISEASE

Eman Gadu[1,2], Amro Hashish[1,3], Mostafa M.S. Shelkamy[1,4], Maria Chaves[1], Onyekachukwu Osemeke[1], Mariela E. Srednik[5], Yuko Sato[1] and Mohamed El-Gazza[1]

[1]Iowa State University, Department of Veterinary Diagnostic and Production Animal Medicine, 1809 South Riverside, Ames, IA 50011.
[2]Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.
[3]National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza.
[4]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.
[5]University of Wyoming Department of Veterinary Sciences, Laramie, WY 82070, USA.

ABSTRACT

Spotty liver disease (SLD), a reemerging infection caused by *Campylobacter* species, with increased incidence in cage-free commercial layer hens, resulting in increased mortality and reduced egg production. Recently, *Campylobacter hepaticus (C. hepaticus)* has been identified as a key pathogen responsible for SLD. The laboratory diagnosis of SLD primarily relies on isolating C. *hepaticus*, a process hindered by the bacterium's fastidious nature and the requirement for time-consuming and specialized culture conditions. Molecular diagnosis using quantitative real time PCR (qPCR) overcomes these limitations and offers a more sensitive and specific alternative. However, the existing qPCR assay using a DNA-binding dye chemistry, suffers from non-specific binding to any dsDNA in the sample, which could potentially lead to an increased incidence of false-positive cases. In this study, we present the development of a more specific TaqMan probe-based qPCR assay targeting the glycerol kinase gene of C. *hepaticus*. This assay demonstrated excellent analytical specificity and sensitivity, with a detection limit of 5 copies/µL and a PCR efficiency of 95.15%. Additionally, it exhibited 100% diagnostic specificity and sensitivity. Furthermore, probe-based PCRs are the most commonly used type of diagnostic PCR assays and are much better suited for routine diagnostics compared to other types of PCRs. In conclusion, the newly developed assay represents a significant advancement in the accurate and efficient diagnosis of SLD caused by C. *hepaticus* directly from clinical samples.



SALMONELLA VACCINE BBS 866 REDUCES CECAL COLONIZATION AND SYSTEMIC DISSEMINATION OF DSC ENTERITIDIS IN CHICKENS

Samuel J. Whelan[1,a], Bradley L. Bearson[2], Maya P.N. Encinosa[1,a], Durga P. Neupane[1,2,a], and Shawn M.D. Bearson

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OBJECTIVE

Salmonella enterica serovar Enteritidis (S. Enteritidis), a common cause of human foodborne illness globally, frequently colonizes poultry without causing signs of disease. Recently, S. Enteritidis with decreased susceptibility to ciprofloxacin (DSC), a critical antibiotic prescribed for complicated human infections, has emerged in broiler chickens with the *gyrA* gene encoding a D87Y modification. In the current study, vaccination with the cross-protective, cross-species USDA BBS 866 *Salmonella* DIVA vaccine was evaluated for reduction of DSC S. Enteritidis in broiler chickens.

METHODS

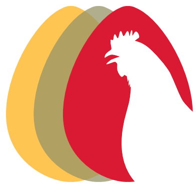
One-day-old broiler chicks were vaccinated at one day of age via aerosol spray and booster vaccinated at two weeks of age via water administration; mock-vaccinated chicks were similarly administered phosphate buffered saline. At 5 weeks of age, chickens were orally challenged with DSC S. Enteritidis strain FSIS12211648. Chickens were randomly selected at 7- and 14-days post-inoculation (dpi) for qualitative and quantitative bacteriology of the cecum (site of prolonged and high levels of *Salmonella* colonization) and the spleen and bone marrow (two sites that indicate systemic dissemination) to determine prevalence and colonization load.

RESULTS

Prevalence of DSC S. Enteritidis (i.e. +/-) was significantly decreased in the vaccinated chickens for the cecum (14 dpi), spleen (7 and 14 dpi), and bone marrow (7 dpi) compared to the mock-vaccinated chickens. Quantitative analysis revealed significant reduction in colonization levels of DSC S. Enteritidis in the vaccinated chickens at 7 dpi for the cecum (2.7 logs), spleen (2.4 logs) and bone marrow (0.4 logs) and at 14 dpi for the cecum (1.6 logs) and spleen (1.6 logs) compared to mock-vaccinated birds.

CONCLUSIONS

Vaccination with the USDA BBS 866 Salmonella vaccine significantly reduced cecal colonization and splenic dissemination and prevented dissemination to the bone marrow by DSC S. Enteritidis in broiler chickens, indicating a promising intervention for preventing food product contamination by DSC S. Enteritidis.



SCREENING NOVEL BIOCIDES FOR RAPID KILLING OF SALMONELLA ISOLATES OF CONCERN

Maya Encinosa[1§], Lucas Thompson[1§], William M Hart-Cooper[2], Bruno Estefano Quintanilla Florian[2], Roberto de Jesús Avena-Bustillos[2], Keith Meeks[3], Torey Looft[1], Shawn M.D. Bearson[1]

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ABSTRACT

Salmonella is a human foodborne pathogen that frequently colonizes animals sub-clinically, resulting in contamination of food products. The USDA Food Safety and Inspection Service recently proposed *Salmonella* as an adulterant in specific poultry products and selected serovars I 4,[5],12:i:-, Enteritidis, Typhimurium, Hadar, and Muenchen as key performance indicators to monitor progress towards the reduction of *Salmonella* in food products. To decrease *Salmonella* contamination in processing facilities and poultry products, disinfectants and sanitizers are employed including peroxyacetic acid (PAA), Wexcide, Virkon, and Virocid with recommended dilutions of 50-2000 ppm, 1:128 (502 ppm), 1:100 (2291 ppm), and 1:170 (2093 ppm), respectively. With the goal of identifying alternative sanitizers with improved efficacy and/or safety, this investigation screened 44 novel biocides for rapid killing of *Salmonella* strains of concern. Two novel biocides (A6 and A16) were evaluated (along with PAA, Wexcide, Virkon, and Virocid) for their effectiveness against five serovars of *Salmonella* enterica representing serogroups B (Typhimurium and I 4,[5],12:i:-), C (Infantis and Hadar), and D (Enteritidis). Overnight cultures of *Salmonella* were diluted to ~1×10^5 CFU/ml and added (equal volumes) to serial two-fold dilutions of each biocide in tap water. After 30 seconds of exposure, reactions were serially diluted (10-fold to 1 X 10-8), plated on LB (Lennox) agar, and grown overnight to determine CFUs present after biocide exposure compared to water-only control samples. A 30 second exposure to novel biocides A6 and A16 at 50-100 ppm resulted in 100% killing of the five serovars tested. Killing of the five *Salmonella* serovars was observed at 2 ppm of PAA, 161-322 ppm of Wexcide, 286-573 ppm of Virkon, and 111 ppm of Virocid. The efficacy of novel biocides A6 and A16 at low concentrations suggests promising alternative compounds for disinfection of food products and sanitization of food processing surfaces.

EFFICACY OF NOVEL COMPOUNDS WITH BIOCIDAL ACTIVITY AGAINST *CAMPYLOBACTER*

Lucas Thompson[1,2], Maya Encinosa[1,2], Roberto de Jesús Avena-Bustillos[3], Bruno Estefano Quintanilla Florian[3], William M Hart-Cooper[3], Keith Meeks[4], Shawn M.D. Bearson[1], Torey Looft

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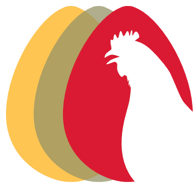
ABSTRACT

As the leading cause of bacterial gastroenteritis in humans worldwide, *Campylobacter* infections are a public health concern. The CDC estimates 1.5 million people become infected with *Campylobacter* every year in the United States alone. *Campylobacter* is widespread in poultry and contamination of food products during the production process increases the risk of human infection. Biocides are an effective way to reduce *Campylobacter* contamination of surfaces and food products. Some currently available biocides, such as peroxyacetic acid (PAA), have safety concerns including respiratory irritation and skin, eye, liver, and kidney damage. PAA is not permitted for washing poultry in Europe and the EU has banned imports of PAA washed poultry, a standard US farming practice. This investigation aimed to test the efficacy of novel biocides to rapidly kill *Campylobacter* sp. as an alternative to PAA. *Campylobacter jejuni* 11168 was grown overnight in Mueller-Hinton broth. Cultures were diluted to ~2 x 10^5 colony forming units (CFU)/ml. Novel biocides (A6, A16, and 113A), as well as PAA, were each serially diluted 2-fold, and the bacterial inoculum was added to each dilution. The concentrations tested ranged from 5000 to 0.15 ppm. After 30 seconds of exposure, 10µL of each reaction was added to 90µL of Dey-Engley neutralizing broth and allowed to neutralize. 10µL of the reaction was plated on Mueller-Hinton agar and incubated at 42°C for 48 hours in microaerophilic conditions (85% N2, 10% CO2, and 5% O2) to determine CFUs. All biocides showed killing of *Campylobacter* sp. Growth inhibition was observed for 113A, A16, A6, and PAA at and above 313 ppm, 39 ppm, 20 ppm and 10 ppm, respectively, suggesting that the tested novel biocides could be an alternative product for reducing *Campylobacter* sp. load. Additional testing is needed to determine how these compounds perform under conventional conditions and with other pathogens.



NANOPORE SEQUENCING: REAL-TIME GENOMIC SOLUTIONS FOR RAPID DIAGNOSTICS OF AVIAN FOREIGN ANIMAL DISEASES

Maria Chaves[1], Amro Hashish[1,2], Mostafa Shelkamy[1,3], Eman Gadu[1,4], Swathi Nadendla[1], Yuko Sato[1], David Suarez[5], Mohamed El-Gazzar[1]

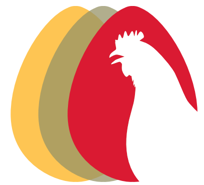
[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University
[2]National Laboratory for Veterinary Quality Control on Poultry Production, Giza, Egypt.
[3]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.
[4]Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.
[5]US National Poultry Research Center, Agricultural Research Service, US Department of Agriculture, Athens

ABSTRACT

In veterinary diagnostic laboratories (VDLs), standardized and time efficient protocols are essential for the rapid detection and characterization of animal infectious diseases. These protocols provide timely and reliable answers to veterinarians and producers in the field, enabling them to stablish effective plans of action to manage outbreaks. This is particularly crucial for avian foreign animal diseases (FAD), such as Avian Influenza (AIV) and Newcastle (NDV), which are highly significant for the poultry industry and require swift containment measures. In such cases, understanding the genomic features of these viruses is vital for predicting their genotypes (epidemiological relevance) and pathotypes (virulence).

At Iowa State VDL, the current diagnostic workflow for AIV and NDV relies on a multi-step process for complete identification and characterization of these viruses. Initially, an RT-qPCR is performed as a screening test, and preliminary characterization (identifying markers for highly pathogenic Avian Influenza and virulent Newcastle) can be achieved. Next, samples are submitted to either Sanger or Illumina sequencing for complete characterization. For NDV genotyping, after RT-qPCRs are completed (for fusion and matrix genes), an initial fusion (F) gene-PCR and Sanger sequencing are conducted. The resulting sequences guide the following primers needed to generate the complete segment of the F gene, which will provide genotype and pathotype information from the samples. The entire workflow typically takes between 2-3 weeks to be completed, and it is not a straightforward process, since it will depend on the strain of NDV that is present in the samples (with primers varying between genotypes).

Nanopore sequencing, combined with a universal amplification PCR for each virus (AIV and NDV) overcomes the limitations of traditional methods. Its unique real time generation and analysis of sequences, integrates the identification and characterization processes on a single platform, reducing the turnaround time to a few hours. Amplicon-based enrichment allows for significant increase in viral concentration from clinical samples, which paired with Nanopore sequencing, delivers a streamlined and time-efficient workflow. Our group is actively working on optimizing Nanopore sequencing as a frontline diagnostic tool for avian FAD, refining every step in the workflow, to ultimately deliver a sample-to-answer protocol, that allows for rapid and accurate diagnosis of AIV and NDV.



THE UNSEEN CHALLENGE: DISTINGUISHING PATHOGENIC FROM NON-PATHOGENIC *AVIBACTERIUM PARAGALLINARUM* IN COMMERCIAL LAYERS

Mostafa M.S. Shelkamy[1, 2], Amro Hashish[1, 3], Eman Gadu[1,4], Mariela Srednik[5], Callie Fay[1], Onyekachukwu Osemeke[1], Maria Chaves[1], Nubia R. Macedo[1], Stephan Schmitz-Esser[6], Qijing Zhang[5], Yuko Sato[1], Mohamed El-Gazzar[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA.
[2]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.
[3]National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza.
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[5]Department of Veterinary Microbiology & Preventive Medicine, College of Veterinary Medicine, Iowa State University, 1800 Christensen Drive, Ames, Iowa 50011.
[6]Department of Animal Science, Iowa State University, Ames, IA 5001.

ABSTRACT

Infectious coryza (IC) is a bacterial respiratory disease in chickens caused by the primary pathogen *Avibacterium paragallinarum* (AP). Once AP is introduced into a naïve flock, several noticeable symptoms, such as facial swelling and a decline in egg production, are typically reported. Although the clinical picture is clear, a definitive laboratory diagnosis is still essential for disease confirmation. Recently, we observed multiple naïve healthy layer flocks that had neither been exposed to IC nor received IC vaccination, yet tested positive using the current IC-specific qPCR assays. We could not explain this phenomenon until we isolated AP isolates from some of these naïve flocks and did whole genome sequencing (WGS). The WGS analysis revealed significant genetic differences between these new AP isolates and the classical pathogenic AP (pAP) isolates. Consequently, these isolates were preliminarily dubbed "non-pathogenic *Avibacterium paragallinarum*" (npAP). From this point, we developed our hypothesis and aimed to achieve three main objectives: 1. Develop and validate differential qPCR assays, 2. Investigate the incidence of npAP in the U.S. layer industry through a surveillance study, 3. Investigate the pathogenicity of npAP strains and their potential protection against pAP via a live bird challenge study.

We made clear progress as follows: Differential qPCR assays were developed and validated, demonstrating the ability to differentiate between pAP and npAP in 85% of cases, with perfect sensitivity and high specificity. Surveillance revealed that 35% of naïve healthy layer flocks across various sites in different states (28/80) were npAP-positive, complicating the current IC-specific qPCR assays, as they are unable to distinguish between pAP and npAP strains. To assess the pathogenicity of npAP strains, naïve laying hens were challenged with seven distinct npAP strains, alongside a positive control group challenged with pAP of serotype C and a negative control group. There were no significant differences ($P > 0.05$) in the mean clinical score between the seven npAP groups and the negative control group, while the pAP group had a significantly higher score ($P < 0.05$) than all others. Upon subsequent exposure to the challenge strain (serotype C AP), two groups pre-challenged with npAP had clinical scores that were numerically lower than other npAP groups and not statistically different from the positive control group. Under the conditions of this study, the assessed npAP strains were confirmed to be non-pathogenic to naïve chickens. The clinical scores observed suggest pre-exposure to certain npAP strains may confer some protection against pAP of serotype C. Further studies are needed to validate these findings under commercial field settings.

IOWA STATE UNIVERSITY

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Mail: U.S. Department of Agriculture Office of the Assistant Secretary for Civil Rights, 1400 Independence Avenue SW, Washington, D.C. 20250-9410; or Fax: 833-256-1665 or 202-690-7442; or Email: program.intake@usda.gov.

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