

# The Poultry Informed Professional

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#### PIP Newsletter Follow-up with Dr. Grazieli Maboni – Castellaniella Research

Since I started as a faculty at PDRC in March 2023, I have been enthusiastic about working on a previously unrecognized bacterial pathogen of poultry called *Castellaniella*. Since 2018, *Castellaniella* has been repeatedly isolated from broiler breeders presenting a variety of clinical presentations.

Here are some details of the *Castellaniella* project that will be performed at PDRC in collaboration with Dr. Jenny Nicholds.

**What is the problem?** There is only one case report of *Castellaniella* causing disease in animals. This case report describes *Castellaniella* as the primary cause of death of small lagomorphs (daurian pika), presenting suppurative inflammation and abscesses in internal organs. To date, there are no documented reports of *Castellaniella* associated with clinical disease in avian species, so it has been previously unrecognized as a poultry pathogen. However, at PDRC, we have been isolating *Castellaniella* repeatedly from flocks of broiler breeders presenting with tenosynovitis, femoral head necrosis, pericarditis and/or swollen wattles with submandibular edema. Since the initial case in 2018, PDRC has observed 20 additional cases with *Castellaniella* isolations across 4 different integrators. Because of the fastidious nature and/or unavailability of molecular tools to identify *Castellaniella*, it may be commonly overlooked in diagnostic labs, specifically those which do not receive significant poultry species submission.

**What we aim to do?** We first want to generate basic knowledge about *Castellaniella* to set the foundation for a future project to exam the *in vivo* pathogenesis in chickens. For that, we will start by sequencing the whole genomes of the isolates obtained from clinical cases in broiler breeders at PDRC and then provide a comprehensive genetic characterization of such genomes. We will also investigate the antimicrobial susceptibility profile based on the minimum inhibitory concentration of antimicrobials.

Last but not least, as there are no specific diagnostic tools for *Castellaniella*, we will develop a Real-Time quantitative PCR assay to rapidly and reliably detect *Castellaniella* from clinical and environmental samples.

**What is the potential value of this project?** This work will help with the design of antimicrobial treatment guidelines for clinical cases of *Castellaniella*, and the RT-qPCR assay will direct effective diagnosis and timely therapeutic and management interventions. This work will also set the foundation to create a challenge model to examine the *in vivo* pathogenesis of *Castellaniella*.



Presented is a photograph of pure colonies of *Castellaniella* isolated on 5% sheep blood agar after 48h of incubation. Swabs were collected from lesions during post-mortem evaluation of affected broiler breeders. Identification of the genus *Castellaniella* has been performed by 16S rRNA gene sequencing based on the Sanger method.

## > Focal Duodenal Necrosis

#### Focal duodenal necrosis (FDN) - the knowns and unknowns of the disease Yu-Yang Tsai, PhD student, Department of Population Health, University of Georgia

Focal duodenal necrosis (FDN) is an intestinal disease of table egg layers, which is one of the top five concerning diseases of the table egg layer industry (1). The economic impact of FDN is associated with a decrease in egg case weight and a drop in egg production. Affected chickens may or may not show subclinical or non-specific symptoms such as lower body weight and pale comb (2). Although FDN was first described in 1996, the etiology of the disease has not been fully elucidated. Some studies have associated FDN with *Clostridium* species (3, 4). In 2016, Franca et al. reported an association between beta2-positive *C. perfringens* type A with FDN in egg layers in the United States (4). A challenge experiment was conducted to reproduce FDN using different *C. perfringens* isolates and duodenal homogenates obtained from FDN lesions. However, the challenge failed to reproduce the characteristic macroscopic and microscopic lesions which are typically found in birds afflicted with FDN but lesions consistent with enteritis were observed (5).

In May, our paper "Laser Capture Microdissection, Culture Analysis, and Bacterial Sequencing to Evaluate the Microbiota of Focal Duodenal Necrosis in Egg Layers" was accepted by Avian Diseases. In the study we used laser capture microdissection (LCM) to excise bacteria-containing lesions from FDN affected birds, followed by 16S rRNA gene sequencing for bacterial identification. Bacterial sequencing analysis revealed no consistent or single bacterial species as a causative agent from FDN samples. However, analysis of the relative phylum abundance revealed differences in the duodenal microbiota between layers with FDN and healthy birds. Although there were no statistically significant differences, the median percentage of Firmicutes in the control FDN-negative group was 76.6%, compared to 36.1% in the FDN-positive group. The median percentage of Proteobacteria in the FDN-negative control group was 19.5%, compared to 37.5% in the FDN-positive group (Figure 1). Other bacterial phyla including Actinobacteria and Bacteroidetes did not show numerical or statistically significant differences between FDN and control samples. These findings revealed differences in microbial composition between FDN-positive and FDN-negative control samples.

The lesion samples subjected to enrichment for bacterial detection generated 30 aerobic and 17 anaerobic bacterial isolates from Tryptone Soy Agar (TSA), MacConkey and blood agar plates. 16S

PCR identified 39/47 isolates as *E. coli*. 16S rRNA Sanger sequencing of the remaining 8 isolates, identified two isolates as *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, while six isolates could not be identified. PCR for *E. coli* virulence genes identified 21/39 (53.8%) *E. coli* isolates as avian pathogenic *E. coli* (APEC)-like. While additional analysis for 19 *E. coli* virulence genes associated with intestinal disease strains including inflammatory bowel disease (IBD) found 11/39 (28.2%) isolates harbored more than 10 virulence genes. Overall, *E. coli* from FDN lesions also appear to possess multiple virulence genes associated with IBD. Among these genes, *fimH* (97.4%), *dsbA* (94.9%), *eaeH* (92.3%), and Microcin mH47 (94.9%) were highly prevalent in our *E. coli* isolates. Other virulence genes such as Colicin B (46.2%), Ia (41.0%); Microcin mV (53.8%), and mM (48.7%) were prevalent in more than 40% of *E. coli* isolates (Figure 2). Although a comparative genomic study of *E. coli* isolates from IBD patients did not identify IBD-specific *E. coli* genes, the results from our research still provide insights that there might be some correlation between *E. coli* isolates from FDN lesions and those associated with IBD (6).

This summer we continued to examine factors responsible for FDN and have conducted two challenge experiments in an effort to replicate FDN in commercial layers. First trial was a small-scale study, using 4 different bacterial cocktails including *Clostridium perfringens, E. coli, Enterococcus faecium, Gallibacterium anatis*, and *Clostridium colinum*. In the second trial we narrowed the potential candidate pathogens down to *Clostridium perfringens, E. coli* and *Clostridium colinum*. We have observed lesions in the duodenum that resemble the presentation of FDN. However, we are currently in the process of analyzing the data. Stay tuned for the upcoming IPPE/IPSF and AAAP meetings where we will be presenting this data.



Figure 1. Percentage of the total count for each Phylum from FDN positive lesions. The mean and median of percentage of Firmicutes in control group are 58.2% and 76.6%, respectively; in FDN positive group, those are 36.1% and 34.1%. In Proteobacteria, the mean and median of percentage of Proteobacteria in control group are 19.5% and 10.7%, respectively; in FDN positive group, those are 37.5% and 30.4%.



Figure 2. Results of PCR related to genes that are associated with inflammatory bowel disease and their prevalence among the *E. coli* isolates.

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# Interesting Topics presented at the 58th National Meeting on Poultry Health, Processing, and Live Production Highlights

Reviewed by the Master of Avian Medicine Candidates

#### Management of Runting Stunting Syndrome – Dr. Connie Mou

A highlight of the National Meeting on Poultry Health, Processing and Live Production was Dr. Connie Mou's presentation on management of Runting Stunting Syndrome (RSS). Runting stunting syndrome is a viral enteric disease of chickens that often presents with delayed growth, decreased body weights, and increased feed conversion ratios in commercial broilers. Runting stunting syndrome been present in the commercial industry for many years, and integrators typically rely on factors such as vaccination, biosecurity, and management to control this disease. During Dr. Mou's presentation, she discussed the impact adequate management has on reducing stress, maintaining bird comfort levels, and maintaining overall health in flocks with stunted birds. Factors such as temperature, feed and water consumption, and flock distribution should be evaluated in flocks with cases of RSS. Temperature impacts chick behavior and cycling patterns due to its ability to alter bird comfort levels. Birds cycle properly and are comfortable with proper environmental temperatures but experience disruptions in cycling with improper temperatures (i.e., cold stress). Temperature is crucial is managing commercial birds as chicks are unable to regulate their body temperature which is of even more importance in cases of RSS. Birds should be placed in environments that are well heated with proper circulation throughout the house which can be achieved with the use of circulation fans. Circulation fans operate by gently removing hot, dry air off the ceiling and down towards the floor and are to reduce temperature stratification, improve temperature uniformity, and conserve energy. These fans are also able to reduce hot spots under radiant heaters, leading to more uniform litter moisture profiles across the width of the house, drier litter, and improved paw quality. With the usage of circulation fans, chicks can spread more evenly following placement because the floor temperature is more comfortable to the birds. As a result, integrators are also able to maintain equal access to feed and water, avoid wasting of floor space, reduce poor litter quality (caking), and prevent impaired access to feed and water. In addition to proper house temperature, access to water is essential in managing cases of RSS. Birds often travel from hatcheries from great distances and should be encouraged to drink upon farm arrival to prevent severe dehydration. To improve water consumption, many commercial integrators place paper under the drinker lines to attract birds to increase water consumption. As a result, the birds also consume increased levels of feed, leading to improved first week body weights, decreased mortality in the first week, and an overall improvement in first week performance. Feed consumption also plays an important role in maintaining bird comfort levels. Chick papers are often placed under feed lines to encourage and increase access to feed for the birds. After discussion of methods to reduce bird stress levels, Dr. Mou reviewed practical methods to monitor bird comfort on commercial farms. Water meters were found to be a great tool for measurement of daily flock water consumption. The ultrasonic water meters are an adequate and affordable device that can be used to measure and observe flock water consumption. A direct relationship exists between feed and water usage in birds which can also provide growers insight into their flocks' feed consumption. In conjunction with measurement of water consumption, water meters provide a historical database from previous flocks' water consumption patterns that can be used to predict changes in flock health based on these past trends. Although the use of water meters is an adequate tool to measure water consumption, the quality of this test can be further improved by monitoring consumption on a minute-by-minute basis. This analysis provides a more detailed investigation of water consumption throughout the day and

allows growers to identify changes in water consumption that may have otherwise been missed with reliance on daily water consumption monitoring.

In conclusion, proper management is essential in handling birds with RSS. When birds are raised at the proper temperatures, this improves cyclical behavior of birds, results in increased feed and water and consumption, and overall, more uniform distribution of birds across poultry houses. If we are better able to maintain overall bird distribution, it facilitates management of flocks with poor uniformity as demonstrated in cases of Runting Stunting Syndrome. **Summary Provided by: Tiffani Allen** 

## Broiler Breeder Fertility & Hatchery Current Issues – Dr. Jeanna Wilson

Dr. Jeanna Wilson, Professor in the Department of Poultry Science at University of Georgia, spoke on current issues with broiler breeder fertility. Her talk focused on management of roosters as a means of influencing fertility. Goals of male management relating to fertility include improving sperm mobility and guality, improving libido and ability to complete mating, and increasing the number of males that sustain semen production to 60 weeks of age. Male fleshing and body weight play a significant role in a rooster's reproductive capacity. Poor implementation of feed restriction can result in overweight males that physically cannot complete mating and underweight males that end up being culled. Aggressive feed restriction can also result in testicular regression or atrophy resulting in decreased semen production. Target weight for testes at 30 weeks is 35-50 grams with a testes to body weight ratio greater than or equal to 1. Addressing foot and leg issues through culling or addressing predisposing causes will help improve the number of roosters successfully mating. Environmental factors can also impact mating and fertility. With excess heat and humidity, especially over 85 degrees Fahrenheit, semen production will decrease and will not return until conditions cool off. Cockerels are often raised on the fan side of houses, where it is warmer, which exacerbates the effects of heat stress. Most breeder diets are formulated for hens and fed to hens and roosters, but there are many ingredients such as antioxidants that can help protect spermatozoa from peroxidation if included in the diet. Proper maintenance of number of males in a house is also important in sustaining semen production through the life of a flock. A ratio of 8.5-9.5 males/100 hens is appropriate, higher ratios risks hens moving to slats to avoid mating. Mating activity decreases with age and additional of spike males around 40 weeks can help increase overall mating frequency in hens. Dr. Wilson's talk highlights that addressing fertility issues in broiler breeders requires holistic management of males to optimize mating and production of quality semen.

#### **Summary Provided by: Jason Sousa**

# Infectious Laryngotracheitis Vaccination: Past, Present and Future – Dr. Maricarmen Garcia

Dr. Garcia demonstrated throughout her presentation the importance of genotyping ILT cases because it allows for better control strategies against future outbreaks, determining if outbreaks are field or vaccine origin as well as understanding the evolution of circulating viruses. Although there are benefits for avian health there is also the concern, because ILT is reportable and reporting genotypes other than vaccine related strains could potentially have trade implications. The descriptions of the nine ILT genotypes goes as following: I – USDA Challenge strain, II- TCO Vaccine, III- Virulent strain closely related to TCO, IV – CEO

Vaccine, V – Virulent strains closely related to CEO vaccines (present independent of CEO use), VI – Virulent field strains not related to vaccines and genotypes VII-IX – reported in the 1980-90's with backyard flocks. While genotyping, using multiplex PCR and minION sequencing, she concluded that there has been an increased incidence of broiler breeder and broiler cases involving the genotype VI viruses over the past several years. To further characterize the virulence of this genotype, experimental studies were performed inoculating separate groups of birds with genotype VI isolates from 2022 and 2004 both with intratracheal and ocular inoculation. Between the two groups there was no statistically significant difference in viral replication, however the virus from 2022 had a significant increase in clinical signs by day five, compared to the 2004 virus isolate. Increased mortality was also noted with the 2022 virus when inoculated via the intratracheal route. Dr. Garcia also reviewed the efficacy of vaccine strategies staging that CEO leads to a protection against clinical signs, blocking challenge virus replication and transmission, but can lead to vaccine reactions affecting flock performance if it is not applied with high coverage. TCO vaccine and HVT vaccine strategies allowed for the reduction of clinical signs as well as challenge virus replication, but they do not block transmission of the challenge virus. Combinations of priming with recombinant vaccines and then using CEO and TCO live attenuated vaccines has also been found to be efficacious. Future implications include built in maintenance of safety standards regarding recombinant vaccinations, exploring new molecular editing tools to modify live attenuated vaccines as well as exploring new ways to safely enhance ILT mucosal immunity as sites of viral entry to block viral shedding. **Summary Provided by: Cole Taylor** 

#### **Useful Links**:

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