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**Poultry Diagnostic
and Research Center**

College of Veterinary Medicine

UNIVERSITY OF GEORGIA

► Entrance Interview with Dr. Grazieli Maboni

Interview Provided by: Ashley Hallowell

- **Tell us about your background and how you got into poultry industry/microbiology research? What brought you to Athens?**

In high school, I heard about Alexander Fleming who discovered penicillin in London and presented a project on this. I contacted the mycology lab from the Federal University of Santa Maria, in Brazil, to look at a *Penicillium* growth on an agar plate and the lab was kind enough to show me around. From here, I knew I had an interest in lab work related to microbiology and antibiotics/antifungals, so I decided between pharmacy or veterinary medicine. I applied to veterinary school and since my first month in vet school I worked in that mycology lab and found my passion there. After three years in the mycology lab, I transitioned to work in the bacteriology lab. This was another eye-opening experience for me, and this is where I became interested in bacteriology as well. I received a scholarship to work in Athens at the diagnostic lab. This was a life changing opportunity. I began to understand how the diagnostic system in a developed world worked. I met Margie Lee through a bacterial pathogenesis class and she mentored me while I worked in her lab analyzing the gut chicken microbiome. I returned to Brazil for a Masters program in microbiology, and went to Nottingham, in England, to pursue my PhD in microbiology. Interestingly, I was thinking about Athens and coming back to UGA, so at the completion of my PhD, I knew I wanted to sit for microbiology boards and get further diagnostic skills, therefore I pursued a three-year residency program in microbiology at the Athens diagnostic lab. I finished the residency program and was offered the opportunity to work as a faculty in Guelph, Canada, where other legends of the bacteriology field came from. Once I saw the job position with PDRC, I was instantly excited about the opportunity to come back to UGA!

- **What are your main research and teaching goals at UGA PDRC?**

For research goals, I want to work on developing new tools for diagnostics, to essentially elevate poultry microbiology diagnostics at the molecular and proteomics level. I want to help the lab gain further accreditation, to work on quality control, and to focus on new diagnostic capabilities and technologies. For basic microbiology research, I am interested on working with the different roles of co-infections (bacterial and viral) within the respiratory system of chickens. I have an interest in the gut-lung axis (microbiota in the gut versus the lung) and how this may impact the development/colonization of certain pathogens. For teaching goals, I want to make microbiology effective for the MAM program. I will be teaching avian microbiology and bacterial pathogenesis. I want to have the avian microbiology class heavily focused on clinical case discussions and brainstorming in a way that we can be comfortable enough to sort out clinical cases together. Essentially, taking the puzzle pieces and putting them together. I want to make the lectures based on clinical cases along with some background information.

- **What motivates you in your career?**

My biggest motivator is working to elevate microbiology and creating a potential career

path for next generations. I want to fuel a field within veterinary medicine. Not many students overall consider the microbiology career path. I want to share my enthusiasm for microbiology and share that potential pathway for students and next generations that are interested in microbiology. And with this, building a structure for the microbiology specialty in veterinary medicine. The more we can attract veterinary microbiologists, the more funding, research, and residency programs will be available. Additionally, I am focused on finding new ways to diagnose or treat animals and reduce the need of antimicrobials whether that be with alternative compounds or management. Veterinarians are crucial in focusing within this area of research since human researchers are not generally focused on answering these questions.

- **What do you hope will be your lasting impact at UGA PDRC?**

I hope to train and inspire vet microbiologists. I would be interested in developing a residency program in clinical microbiology and include poultry microbiology within this residency program. Having new microbiologists focused on poultry would be great. I desire to have a research lab that has a practical application in avian medicine and elevating diagnostics in the poultry field. I am also involved in outreach activities in developing countries. I have received international funding to go to Brazil to teach microbiology and diagnostics in public universities. I have been helping to implement, next generation sequencing there. Overall, I want to assist developing countries to promote microbiology and diagnostics. I hope I can travel to other countries to provide a tool to diagnose bacterial infections and look at antimicrobial resistance. Tackling antimicrobial resistance is a global responsibility and we need all facilities in the world to be able to identify antimicrobial resistance. We know how to work on those cases here in the United States, but this is not the reality everywhere. We can't neglect how antimicrobial resistance is being detected and diagnosed in other places – it is a global perspective.

- **What advice do you have for PDRC student researchers?**

Find your niche! Find what you like doing. Find what inspires you. If it fulfills your heart, you will accomplish a lot of great things. Don't fear trying different things. Another thing to keep in mind, is that things will unfold naturally. If you feel resistance, that it is not in alignment with you and your goals and your values, try finding something else. It could make you happier and make a bigger impact if it aligns with you.

- **What are your hobbies outside of UGA PDRC?**

I like dancing, walking my dog (Popcorn!!!!), and visiting parks

► Interesting Topics at IPPE/IPSF 2023

Summary Provided by: Ashley Hallowell

Assessing *Salmonella* serovar dynamics through broiler processing

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As a leading cause of bacterial foodborne illness, 17% of salmonellosis cases are attributed to broilers. *Salmonella* is diverse with different phenotypes and the serovar is defined by the combination of O and H antigens. For example, serovar Gallinarum is host restricted, while serovar Typhimurium can colonize a wide variety of hosts. *Salmonella* serovars can be filtered down to those that typically are found in poultry and poultry environments, survive interventions in pre-harvest (e.g., vaccination) and processing (e.g., tolerance to PAA, formation biofilms), and can cause human illness. Today, conventional isolation of *Salmonella* involves characterizing a single colony from a positive sample. However, this method will favor the most abundant serovar in that sample (or that which grows best under the conditions used), thus not detecting other serovars that could be present. Previously covered in PIP Newsletter Issue 146, deep serotyping by CRISPR-SeroSeq is a novel, high-resolution sequencing approach can detect and quantify relative frequency of multiple serovars within a sample.

CRISPR-SeroSeq was utilized in this study to determine how interventions in the processing plant may influence the serovar population and *Salmonella* incidence. Paired broiler carcasses at hot re-hang and post-chill along with additional re-hang samples without a paired *Salmonella*-positive-post-chill were evaluated. The main questions to be answered involved: What is the impact of antimicrobial interventions on *Salmonella* serovar complexity? And are certain *Salmonella* serovars able to better survive processing interventions? Samples were collected from broiler processing plants from August to November 2022 through the United States via the FSIS Young Chicken Carcass Exploratory Sampling Program. Re-hang samples showed that there was an average of 1.62 serovars per sample where Kentucky was found in 73.3% of samples, followed by Infantis (33.7%) and Typhimurium (17.4%). For post-chill samples, there was a 1.12 average number of serovars per sample where Kentucky was the predominant serovar (89.5%) and Infantis was not detected. Ultimately, multi-serovar *Salmonella* populations are often found in re-hang samples and rarely in post-chill samples. Therefore, this data demonstrates that processing interventions reduce the overall serovar diversity.

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Stunning and neck-cutting methods impact on the overall blood loss and rate of blood loss of broiler carcasses

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With over 9 billion broilers processed each year in the U.S., blood is a significant by-product of poultry processing. Blood makes up relatively small proportion of overall body composition (8%); however, a poor bleed-out will result in increased wastewater treatment, increased microbial loads, and can cause carcass downgrades. Interestingly, there is little research on the effect of stunning method on blood loss in today's high breast meat yield broilers. The goal of this research was to evaluate blood loss and the rate of blood loss for 3 stunning methods. Male broilers from four flocks at 62 days of age (N=120; BW 5530g), 45 days of age (N=120; BW 3561g), 43 days of age (N=120; BW 3455g), and 43 days of age (N=120; BW 3527g) of age were used in this study. Broilers were randomly assigned to 1 of 3 stunning methods: a five second 120V AC electric stun from beak to vent (AC), a ten second pulsed 25V DC electrical stun from beak to vent (DC), and a 5-minute CO₂ stun (gas for 3 minutes and a hold for 2 minutes – controlled atmospheric stunning). These methods were used as most broilers in the U.S. are electrically stunned (recoverable stun) using a DC water bath prior to neck-cut. However, controlled atmospheric stunning (non-recoverable stun) is gaining popularity due to pressures from consumer and animal activity groups because of the perceived animal welfare benefit.

Prior to stunning, birds underwent an 8-hour feed withdrawal prior to stunning. After stunning, birds received either a one-sided neck cut severing one carotid and jugular, or a two-sided neck cut severing both carotids and jugulars. Carcass weights were recorded at fifteen second intervals for 180 seconds. Rate of blood loss and % blood loss were calculated based on bodyweight loss using a hanging scale. The hypothesis was that initial rate of blood loss will be significantly different across stunning; however, overall blood withdrawal will not be different.

Overall, DC stun resulted in significantly greater blood loss and rates of blood loss than either AC or CAS, while cutting method and treatment interactions were not significant for any time point or flock. At 90s of bleed-out DC stunned birds lost 42% more blood than AC and 46% more than CAS. At 180s of bleed-out DC stunned birds lost 32% more blood than AC and 35% more than CAS. The question was raised about where the blood is going in AC and CAS. It was determined that the AC and CAS had higher viscera weight compared to DC. Ultimately, there is a potential for sub-optimal bleed out in CAS and AC stunning systems which can result in a food safety concern and increased cost of wastewater treatment. These results highlight the need for potential modification of bleed-out and blood collection when changing stunning method in broilers.

► Inclusion Body Disease/Adenovirus Associated Lesions

Study provided by: Silvia Carnaccini, DVM, PhD, DACPV, DACVP

Tissues from 4-week-old broiler breeders (ROSS) were submitted for histopathologic evaluation at the Poultry Diagnostic and Research Center. Clinical history reported the suspect of inclusion body hepatitis in a flock of 12,000 birds and the request to rule out adenovirus associated lesions.

Histology: The hepatic parenchyma was effaced by random coalescing areas of lytic necrosis with loss of architecture, fibrin deposition, hemorrhage, and invasion by moderate numbers of degranulating heterophils, and macrophages. Degenerate hepatocytes surrounding the necrosis contained numerous round, amphophilic to basophilic, intranuclear (IN) virus inclusion bodies that were filling and expanding the nucleus (**Fig. 1**). Multifocally, portal areas were expanded by small foci of extramedullary hematopoiesis and moderate numbers of lymphocytes, and plasma cells. Similarly, the pancreatic acini were effaced by mild multifocal lytic necrosis associated with basophilic IN virus inclusion bodies and mild lymphoplasmacytic inflammation. In the gizzard, the koilin layer was eroded and the mucosal cells lining the mucosal glands, were multifocally degenerating with large basophilic intranuclear viral inclusion bodies (**Fig. 2**). Rare IN basophilic inclusions were also observed within the enterocytes of the small intestine associated with minimal inflammation and mild villi alteration. Sections of cloacal bursas and thymuses were severely depleted of lymphocytes with little to no cortex present and marked lymphocytolysis of the remaining lymphocytes (score 4 for lymphoid depletion) (**Figure 3**).

Auxiliary testing: liver and kidney pools submitted as FTA cards tested positive for adenovirus DNA by polymerase chain reaction.

Conclusions: Inclusion body hepatitis (IBH) is an infectious viral disease of chickens caused by strains of Group I Aviadenoviruses. It is most common in young birds and may lead to high morbidity and mortality. Among Aviadenoviruses, Fowl adenovirus D and E are mostly associated with inclusion body disease, whereas FAdV A has been mostly associated with gizzard erosions. In this case, characteristic IN virus inclusion bodies were present in multiple tissues suggesting systemic infection by FAdV D or E, or less likely, coinfection also with FAdV A. Pathogenicity is enhanced by immunodeficiency, stress, and other comorbidities. In this case, marked lymphoid depletion was observed in the sections of cloacal bursa and thymus submitted. Likely differentials included infectious bursal disease virus (IBDV), chicken anemia virus (CAV), Marek's disease virus (MDV) and others. Further testing for immunosuppressive causes in this case was not pursued.

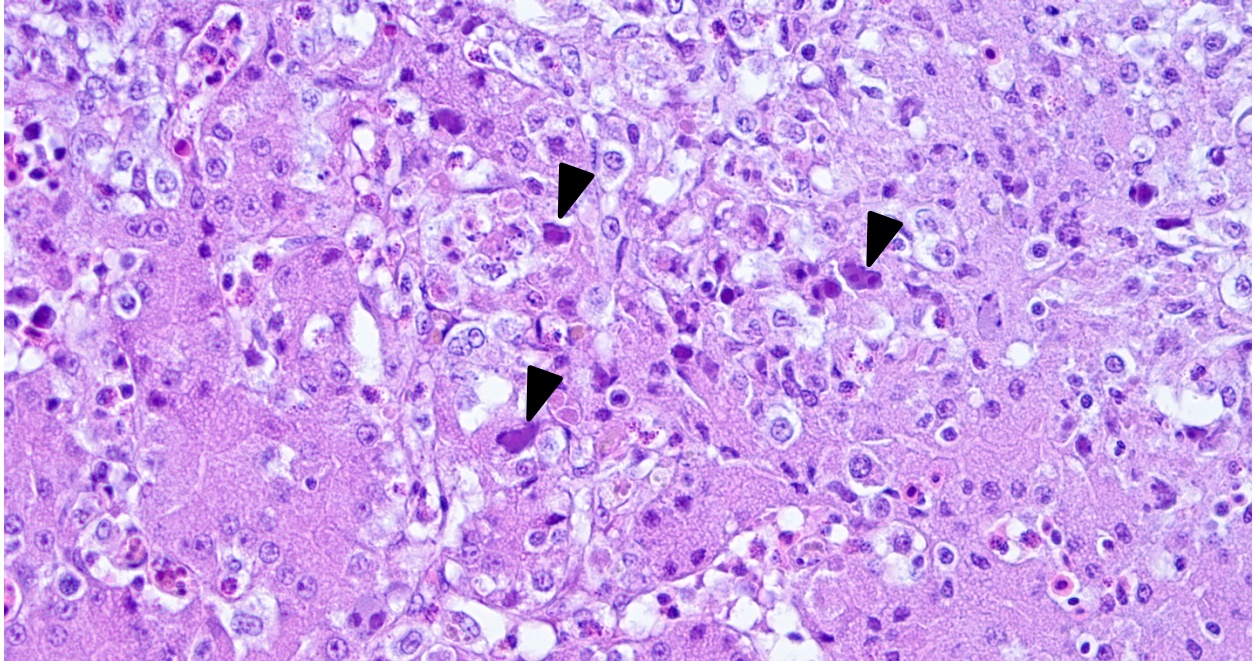


Fig. 1. Liver, 40X. Palisading multifocal areas of lytic necrosis are degenerate hepatocytes with large IN basophilic virus inclusion bodies (arrowhead).

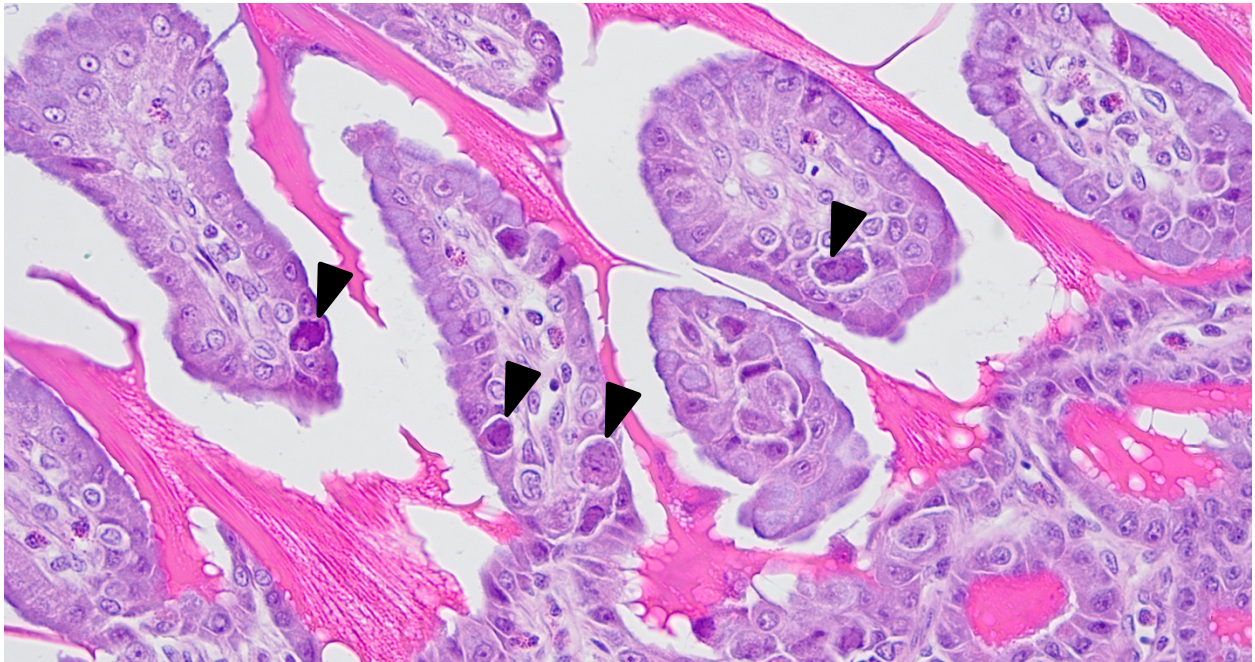


Fig. 2. Gizzard, 40X. Basophilic IN virus inclusions are frequently seen in the epithelial cells of the tubular glands.

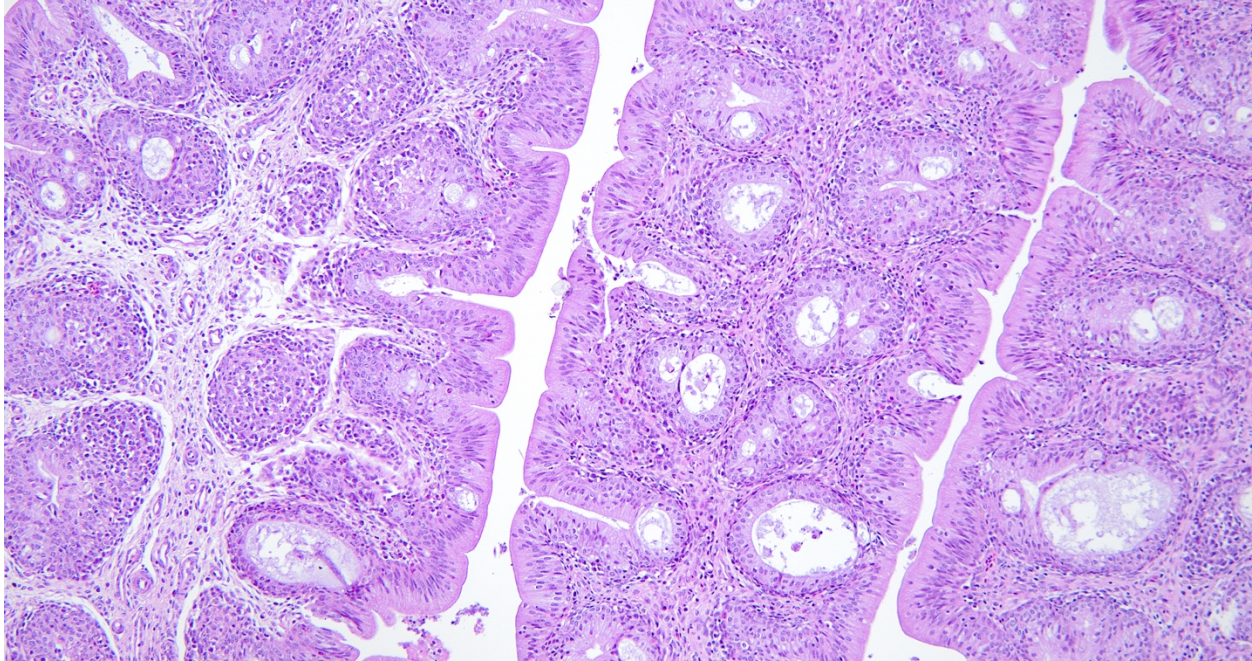


Fig. 3. Cloacal bursa, 10X. There is severe diffuse depletion of lymphocytes and cystic degeneration of the follicles.

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