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Poultry Diagnostic and Research Center College of Veterinary Medicine UNIVERSITY OF GEORGIA

Q & A with Dr. Silvia Carnaccini

Interviewed by Isabella Hannay

Tell us about your background and how you got into Avian histopathology.

I grew up in the heart of Romagna, an Italian region second only to Veneto in terms of poultry production. Just to give you an idea, the rooster with a steel rod is the emblem of the regional flag and often seen embroidered in traditional fabrics and tablecloths! Besides these, I am a 4th generation veterinarian in my family, with my grandpa being a nationally acclaimed avian pathologist thanks to his contribution to the diagnostic and poultry field. Proud of his achievements, I decided to follow his footsteps and become a veterinarian. My path towards avian histopathology, however, did not truly start until my externship at the California Animal Health and Food Safety laboratory (UCDavis) in Tulare, CA. Thanks to Dr. Prasad's passion and knowledge, I fell in love with histopathology!

Do not get me wrong: there are some great pathologists in Italy too, but histopathology, per se, is rarely applied to routine diagnostics and even less so, in poultry. My training continued with my residency in avian medicine and becoming a diplomate of the American College of Poultry Veterinarians. During this time, I firmly established my interest in avian pathology and furthered my learning thanks to exceptional mentors like Drs. Bickford and Stoute.

After my residency, I came to the University of Georgia with the goal of learning how to do high quality research and further improve my preparation in histopathology. Thanks to my dedication, perseverance and not without the support of Prof. Perez (my PhD supervisor), Dr. Jackwood, and the exceptional pathologists in Athens (Drs. Howerth and Stanton) and Tifton (Drs. Ilha and Graham), I was able to complete my training in general pathology and obtain the diploma from the American College of Veterinary Pathologists. Even if my story seems straightforward in its course, it is nothing short of miraculous on how well things worked out. It took determination and sacrifices on my part, but I also owe many people, who during my journey, believed and continue to believe in me.

What are your research interests? What areas would you like to focus on with future research?

Diagnostic allows me to keep in touch with the reality of the poultry world and respond to its tangible needs. That is how I started working on avian influenza virus. When I was a resident at the CAHFS-Turlock laboratory, we received cases of H5 low and high pathogenicity influenza A virus (IAV) in poultry as the 2014-2015 influenza epidemic started in the United States. Since then, thanks to Prof. Perez support, I was able to acquire skills in numerous laboratory techniques applied to molecular virology such as reverse genetics, phylogenetics, and antigenic cartography. These complement my skills in diagnostics and are a necessary foundation for planning and developing my own research program. As I start in my new position, the battle against influenza is far from over, as evidenced by the rise of new HPAIV H5N1 outbreaks across Europe and recently, its detection in wildlife here in the US and poultry in Canada. I already have some ideas that I want to test that can potentially improve the current situation. My research interest also encompasses other neglected pathogens since my concern goes beyond avian influenza. Going forward, I intend to keep my horizons open to build my own area of expertise.

What are you most looking forward to with your new job at PDRC?

Above all, I look forward to working with the faculty and staff that I respect and admire. The PDRC is home to a multitude of excellences in poultry diagnostics, research, and teaching and I am honoured to be part of this family. I also cannot wait for opportunities of collaboration with the USDA Southeast Poultry Research Laboratory across the street, as well as the other UGA departments and colleagues from other institutions.

What is your favourite disease to look at under the microscope?

It is so hard to choose! If I really must pick, I am going to say amyloidosis because the Congo red stain generates that mesmerizing apple green birefringence effect under polarized light.

How is the US poultry industry different to the Italian poultry industry?

I would say that both industries have in common the same goal: to provide an affordable, healthy, and quality product to consumers with respect to the well-being of the animals. That said, differences are rooted into

respective consumer cultures, history, geography, and poultry diseases prevalence. Culturally, Italians prefer brown eggs with a red/orange yolk (>12 DSM yolk colour fan), in contrast with the white shelled eggs and yellow yolk that is more common in the US. Italians mostly cook at home and like to make traditional "yellow pasta" such as tagliatelle, cappelletti, ravioli, etc. Also, the eggs in Italian supermarkets are sold at room temperature instead of refrigerated. Specialty products such as capons, old hens (for the broth), and roosters are also commonly found on Italian market shelves. The geography is definitively another major difference between Italy and the USA: the distribution of farms on the territory, the vicinity with other human activities and poultry density are an issue in terms of disease control and biosecurity. Italy is at the centre of major wildfowl migratory routes (as a promontory on the Mediterranean Sea) and therefore seasonal outbreaks of avian influenza are recurrent and devastating.

What has been your favourite histopathology case to work on?

Every case I have worked on has some backstory that endears it to me. It is difficult to pick a favourite! One honourable mention is the case of trichodinosis associated with pathology of the reproductive tract in commercial waterfowl. At the time I was working on investigating some outbreaks of goose venereal disease caused by mycoplasma. This was a great project I worked on in collaboration with Dr. Ferguson. During this time, I received some samples in which the lesions were completely different: countless bell-shaped protozoa with chitinous adhesive discs were anchored to the mucosa of the phallic structures. I almost fell from my chair! It was thrilling to identify those protozoa as trichodina, which are fish parasites and were obviously not present in any avian pathology book. I also had a lot of fun running the scanning electron microscopy and investigating them in the field. This was one of the cases that prompted me to seek out training in general pathology.

What is your approach to teaching histopathology?

The best way to learn histopathology is to experience it. Throughout my career, I have had the opportunity to supervise and teach students at various levels of education and with different approaches. In the case of histopathology, I like to be creative and use a combination of one-on-one slide review, traditional lectures, projects, and case-based presentations. When possible, I use a personalized approach in which I first assess the student's general knowledge and then build on specific areas that need improvement. I also find assignments as a useful tool for practice and developing critical thinking skills as well to evaluate student progress. I like to challenge the students to think outside the box especially when facing complex cases of debatable interpretation. My philosophy of teaching is to provide a safe environment where students are the protagonists of their own learning, valued for their strengths and encouraged to express their own ideas.

How do you see yourself best serving as a valuable resource for the poultry industry?

This academic position allows me to serve the poultry industry in at least three essential ways: poultry diagnostic, teaching, and research. Diagnostic is an essential aid to clinicians and farmers to solve issues in the field. As a double-boarded poultry pathologist, I have acquired the competences necessary to deliver a quality diagnostic service to our stakeholders. Second, the strong teaching component ensures nurturing the knowledge and passion for avian pathology in the future generations of poultry veterinarians. I am committed to train at best the new veterinarians and students, in the name of those mentors that inspired me to pursue this career in the first place. Finally, I intend to develop research projects on subjects that are key to the improvement of the poultry industry and public health. My current field of interest is avian influenza; however, my plan is to expand my research program to other pathogens.

How does being double boarded (ACPV and ACVP) impact your approach to diagnostics and histopathology?

Each board certification is the testament to my commitment and preparation in the fields of pathology and poultry medicine. The pathology training enhanced my knowledge and experience in interpretation of gross and microscopic lesions. On the other hand, the poultry medicine training allows me to dig into the details of poultry diseases and put lesion interpretation into the context of the real-life scenarios. This allows me to better

communicate diagnostic findings to poultry clinicians and stakeholders and orient my research to be more applicable in the field.

What are your thoughts on the future role of technology in the field of histopathology?

The advancement of technology is inevitable! It is already impacting the way we perform histopathology. Pathologists are already involved in leading the change for the most part: The use of digital slides and platforms are already integrated in our teaching and diagnostic routine. We can share information, consult, and even work remotely at the click of our cursor. Moreover, the web is also flooded with programs that aid in reading of pathology slides, quantify immunostaining results, nuclei, and mitotic figures, etc. These are artificial intelligences that can surpass a human's capability and accuracy of interpretation. Without doubt, these are tremendous tools for the pathologists to ease the workload and standardize the results. However, in the hands of amateurs, they could be misused. Who can confirm, interpret, or troubleshoot incongruences in a software histology reading if not a human pathologist? Therefore, classically trained pathologists are always going to be indispensable. More importantly in practice, lesions must be interpreted within the context of an accurate anamnesis, multifactorial diseases, and confounding factors (like in poultry!). I am confident that the role of the pathologists will keep evolving; it will perhaps be facilitated on some aspects of the routine diagnostic workload, but human ingenuity will always be needed to lead the new advances in technology.

What are your interests outside of histopathology?

I like to spend my free time taking care of my kitties (Aurora and Vivi!), cooking and painting! I love to cook handmade pasta and a few family recipes. I treasure cooking for my friends and family and sharing quality moments together.

I also like to paint – I practice a technique called "pouring painting" and it is a lot of fun! I combine liquid acrylic diluted with pouring reagent and use different techniques to spread them on the canvas. The effects are quite unique and impressive. I also try to keep physically active, and I used to play soccer a lot before the pandemic!

Dr Carnaccini obtained her DVM degree from the University of Bologna, Italy in 2012 and her PhD in Comparative Biomedical Sciences from the University of Georgia in 2020. Her PhD research focused on studying the molecular determinants of antigenicity of the H9 subtype of avian influenza virus. She also contributed to study pathobiology and transmission of various influenza A virus subtypes in vitro and in vivo. Dr Carnaccini is specialized in both poultry medicine and general pathology as a diplomate of the American College of Poultry Veterinarians and the American College of Veterinary Patologists, respectively. She is now an Assistant Professor at the Poultry Diagnostic and Research Center, Population Health Department of the University of Georgia. Her appointment includes routine diagnostic for poultry species, teaching veterinary students, graduate students and residents, and development of her own independent research program. Carnaccini's research, currently focuses on avian influenza pathobiology, evolution at the level of the wildlife-poultry interface. Ongoing collaborations include testing of antivirals against SARS-CoV-2 and study the pathology and tissue tropism of SARS-CoV-2 in multiple animal models.



New Technologies Offered at PDRC to Improve Food Safety

Dr. Brian Jordan

Avian infectious bronchitis (IB) is a highly contagious upper-respiratory disease of chickens. The disease results in major economic losses to the poultry industry worldwide due to poor feed conversion, decreased egg production, predisposition to secondary infections and condemnation at the processing plant (1). Infectious bronchitis virus (IBV), a *Gammacoronavirus* in the family *Coronaviridae*, is the causative agent of IB. As of this writing, there are currently 32 different IBV lineages, with multiple variants within each lineage and more lineages proposed (2). Mutations and recombination in the IBV RNA genome results in the emergence of new antigenic variants relatively frequently (3), which often leads to disease outbreaks. Due to the cost of IBV infection, live attenuated vaccines are used in an attempt to control IB in the field. Vaccine selection is based on prevalence and type of IBV circulating in complexes, as well as the potential for vaccine combinations to offer some level of cross-protection against novel IBVs that emerge. In most complexes, 2 IBV serotype vaccines are applied to broiler chicks in the hatchery, though it can range from 1-3 serotypes depending on situation. At the moment, the combination of a Mass-type and GA08-type IBV vaccine is the most common used in the broiler industry in the US (3).

Being that all current IBV vaccines applied in broilers, and the first few rounds of vaccine for broiler-breeder and layer pullets, are live-attenuated, there are a few ways that these vaccines could be rendered ineffective. Much research has gone into vaccine storage, preparation, mixing, and application (4), and all of these components of the vaccination process can be performed incorrectly and render the vaccine itself useless. Through some of these activities, namely storage, preparation, and mixing, the vaccine virus can be destroyed. This is even more prominent during field application of IBV vaccines when these parameters are monitored more loosely and the process is less controlled than in the hatchery. During application, whether it be spray in the hatchery or in the field, there are many components of the process that will inhibit the vaccine virus from reaching chicks or pullets. Incorrect nozzle size, small application volume, too much pressure, too many or not enough nozzles, incorrect spray angles, etc.; all these factors will negatively impact the application of vaccine. All this being said, we vaccinate chickens every day of the year with live IBV vaccine, and we do get vaccine to many of these chickens. Several checks have been developed to evaluate vaccine preparation and handling that can give you some assurance of efficacy. But there is only one way to ensure that the IBV vaccination process was successful, and that is to ask the chicks.

With the recent advances in molecular biology and diagnostics, we have the ability to detect IBV vaccine virus in chicks soon after vaccination. Showing that the vaccines actually infected chicks proves that the vaccination process was successful, but it also gives you information on how many chicks were infected and to what level. Real time reverse transcriptase polymerase chain reaction (qRT-PCR) is frequently used to detect IBV in clinical samples and can also be used in a vaccine take check program. Assays have been developed and published that will detect all serotype IBV vaccines that we have available commercially, as well as those that may be used on a special permit basis. qRT-PCR is rapid, highly sensitive, and at least semi-quantitative, which allows for sampling from multiple locations and yields data on infection rate and level. My laboratory has developed a vaccine take sampling program based on qRT-PCR, which has helped us develop a vaccine standard for many commonly used vaccines.

A good vaccine take program can be accomplished in several ways, mainly differing in sampling strategy. The primary age to sample birds is 5-7 days post vaccination, where 5 days post vaccination will tell you slight differences in application systems and 7 days post vaccination gives you the primary marker for application success. I typically suggest 7 days post vaccination for my program. Samples can either be swabs from the choanal cleft palate or the trachea, or trachea tissue if the birds are being euthanized. Often times choanal cleft swabs are easier to take, especially on 7 day old chicks, and chickens do not have to be sacrificed for testing. The swabs can be placed in any media, but need to be frozen as quickly as possible prior to shipping to the laboratory for testing. I recommend surveying 6 locations at a time, taking 15 samples per location. This gives 90 samples per survey, which fits perfectly on a 96 well qRT-PCR plate. The sampling location can be an individual house on a farm, an individual farm, or a complex. Variables can include hatchery (if a company has multiple hatcheries), hatch/vaccination day, vaccine crew, or vaccine program. I recommend to start small, and then work up to larger comparisons; i.e. take 15 samples from 1 house from 6 different farms that were all vaccinated on the same day

with the same vaccine program. This would provide the baseline data for that vaccination program, including hatchery or crew success. Then, if all looks good, make the next sampling more in depth. Sample 1 house from 6 different farms where 3 farms (or chicks) were vaccinated on 1 day and the other 3 were vaccinated on another. This allows you to check processes on vaccination day between workers or crews to make sure SOPs are being followed. From there you can expand further and compare hatcheries to each other, different vaccine programs to each other (if running a week on, week off vaccine trial comparing serotypes or manufacturers), or compare different serials of the same vaccine if issues are expected.

Once samples are collected and sent to the laboratory, we would run qRT-PCR on them individually using primers and probes specific to the serotype vaccines used. For instance, if your vaccine program was Mass and GA08, we would use Mass and GA08 primers and probes in our qRT-PCR assay to look for those specific vaccines and nothing else. Processing samples individually is critical for this program; knowing how many of the 15 samples you collected are positive for vaccine is a major piece of the vaccine take puzzle. Once qRT-PCR results were analyzed, we would also be able to provide you with Ct values, which equate to relative viral load. In general, a Ct value above 35 is basically negative, Ct values between 30-35 are slightly positive, Ct values between 25-30 are positive, and Ct values less then 25 are very positive. From each sampling, you would know how many chickens were infected and to what level, which you can then use to adjust the vaccination protocol or process, or not depending on the data.

There are two common presentations of data when evaluating vaccine takes. If all samples (or at least most; >80%) are positive, meaning all chickens were positive, and the average Ct value for the sample set is less than 30, then the vaccination was successful and no further action is needed. If very few samples are positive (<50%) and the average Ct value is greater than 32, then the vaccination was poor and adjustments need to be made. This result is often the easiest to diagnose as it is most likely something obvious and can be easily fixed. These are the most straightforward scenarios, and there are nuances to this data, but these presentations are typically the easiest to interpret. The third scenario is when between 40% - 80% of the samples are positive and the Ct values average ~30 but vary widely, from 25-35, that the interpretation is less straightforward. This would indicate that the vaccination process was not perfectly efficient, but also wasn't completely deficient. This dataset leaves more room for possible causes and could be anything listed above (storage, preparation, application, etc.). These results can be the hardest to diagnose as it could be any part of the process. The situation is a little more complicated when multiple vaccine serotypes are used, as you may get a different profile for each vaccine. But the data still follows the same basic rules discussed above, you just have to look at each piece of vaccine data individually.

There are many ways to check IBV vaccination at the point of vaccine application, but only one way to ensure that the process was truly successful. You must determine if vaccines are actually infecting and replicating in chicks, and the only way to do that is with vaccine takes. Partnering with a diagnostic laboratory to evaluate how many chicks were infected with IBV vaccine, and determining the level of infection, shows you conclusively how efficient your vaccination process is and whether changes need to be made. This data will give you confidence that you are doing everything you can to combat IB caused by IBV, and make your processes as efficient as they can be.

References:

- 1. Cavanagh, D. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol. 32:567-582. 2003.
- 2. Valastro, V., Holmes, E., Britton, P., Fusaro, A., Jackwood, M., Cattoli, G., and Monne, I. S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infect. Genet. Evol. 39:349-364. 2016.
- 3. Jackwood, M. and Jordan, B. Molecular Evolution of Infectious Bronchitis Virus and the Emergence of Variant Viruses Circulating in the United States. Avian Dis. 65(4):631-636. 2021.
- 4. Jordan, B. Vaccination against infectious bronchitis virus: A continuous challenge. Vet. Microbiol. 206:137-143. 2017.



Dr. Brian Jordan is from Colquitt County, GA and received his BS and PhD from the Poultry Science Department at UGA. He was a 4-year letterman on the football team at UGA where he played long snapper. His research interests have focused on Infectious Bronchitis Virus, coccidiosis, and improving hatchery cabinet vaccination. When not working with poultry he enjoys hunting, fishing, and buying horses.

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PDRC Diagnostic Lab Test & Fee Catalog

PDRC Diagnostic Lab - Domestic Submission Form

Interesting Topics at SCAD 2022

Reviewed by the MAM Students

Focal Duodenal Necrosis: identification of the potential role of Gram-negative bacteria in intestinal lesions, Dr.Yu-yang Tsai.

A highlight of SCAD was UGA's Dr. Tsai presentation on his research into Focal Duodenal Necrosis (FDN). FDN is a devastating disease to the table egg layer industry and unfortunately the aetiology is still unknown. Dr. Tsai investigated apparent FDN lesions in 42 ethanol-fixed duodenal samples which had been collected from a variety of affected farms. Microscopic analysis revealed heterophilic and lymphoplasmacytic enteritis, luminal fibrinonecrotic exudate and variable numbers of filamentous bacteria within the lesions. This bacteria was collected using Laser Capture Microdissection which was shown in an on-screen video, this was very interesting as I'm sure many members of the audience had not used this technique before. The 16S rRNA gene of the gram-negative bacteria found within the lesions was then sequenced. The results showed, in the relative phylum abundance analysis, Proteobacteria is more abundant in FDN samples than in control samples, but Firmicutes was more abundant in control samples. Similar dysbiotic gut microbiota is found in inflammatory bowel disease (IBD) in humans, dogs, and other mammalian species. One FDN-affected farm was used for further study involving bacteriology and sequencing. The majority of bacterial colonies identified in this study were E.coli and further virulence gene analysis by PCR found 70.2% of isolates examined possessed APEC virulence genes and 93.6% possessed Inflammatory Bowel Disease virulent determents. The research shows that FDN might be a multifactorial inflammatory intestinal disease associated with dysbiosis in duodenum.

Summary provided by Isabella Hannay

Evaluating Salmonella population dynamics in broiler flocks, Dr.Tomi Obe.

Dr. Tomi Obe, a postdoctoral associate in Dr. Nikki Shariat's lab at the PDRC, presented the results of surveillance testing performed to determine the population diversity of Salmonella in broiler flocks. Of particular interest was the emphasis placed on the impact different production systems may have on Salmonella incidence and serovar diversity. The different production systems evaluated included no antibiotics ever (NAE) and no antibiotics of human medical importance (NAHMI). Eighty broiler flocks (20 farms) ranging in age from 3 to 5 weeks were evaluated over a five-week period using boot socks to obtain samples from the poultry house environment. Utilizing methods discussed in the previous edition of the PIP Newsletter, serovar populations within each broiler flock were determined. This surveillance study found that there was no statistical difference in Salmonella incidence between production systems and that 60% of flocks had more than one Salmonella serovar, with serovars Kentucky and Typhimurium being the most frequently detected. While surveillance efforts are ongoing, the initial findings of this study can certainly function as a tool for live production personnel when making decisions regarding their Salmonella control and intervention strategies.

Summary provided by Maggie Thompson.

Novel genes involved in the biofilm formation of Avian Pathogenic Escherichia coli, Meaghan Young

This research study presented by Meaghan Young involves the investigation of Avian Pathogenic Escherichia coli (APEC), which is the etiological agent of avian colibacillosis, a leading cause of morbidity and mortality in the poultry industry worldwide. Biofilm is an important factor for APEC survival and persistence, although much is still unknown about the genes involved in its formation. Therefore, the objective of this study was to identify novel genes involved in the biofilm formation ability of APEC. Using a well-characterized strain of APEC (APEC 380), 15,660 mutants were randomly created using the signature tagged mutagenesis technique and evaluated for decreased biofilm formation. Mutants with a >50% decrease in biofilm formation ability compared to the wild type were sequenced around the transposon insertion to identify 547 putative biofilm formation genes. To determine which genes were most important in APEC, 30 of the identified genes not known to be involved in APEC biofilm formation were analyzed via PCR for prevalence in 109 APEC and 104 avian fecal E. coli (AFEC) isolates. A total of nine genes had significantly higher prevalence (p < 0.05) in APEC than AFEC isolates. The author concluded that the presence of these genes in APEC at a significantly greater rate than AFEC suggests that these genes are important in APEC biofilms and can be used as potential targets for antimicrobials and other therapeutics without disrupting commensal E. coli. Further research will evaluate the importance of these genes throughout different phases of biofilm production.

Summary provided by Roel Becerra

Experiences with Inclusion Body Hepatitis in the Southeast United States

Dr.Eric Shepherd

Summary

Inclusion Body Hepatitis (IBH) has been a consistent issue in the southeast United States (US) for the last few years. There has been a recent shift towards serotype 8b being predominantly isolated in affected broiler flocks but serotypes 5, 8a, and 11 have also been found. The majority of flocks were typically affected between 2-4 weeks of age and have had daily mortality range from minor (0.1%) to very significant (1-2%). Affected broiler integrators have been able to control IBH using various interventions including, but not limited to, autogenous vaccine development, cleaning and disinfection of houses, litter removal and heat treatment, and control of immunosuppressive diseases. Anecdotal information seems to indicate that the companies using autogenous vaccines in their broiler breeders have had a greater amount of success controlling IBH than those companies who did not use autogenous vaccines. While there is still debate if IBH is a result of a primary or secondary pathogen, our integrator questionnaire results and University of Georgia Poultry Diagnostic Research Center (UGA PDRC) submissions suggest that serotype 8b may be a primary pathogen with nearly 83% of submissions being serotype 8b.

Introduction

Inclusion Body Hepatitis (IBH) is caused by a double stranded DNA virus that belongs to the genus Aviadenovirus of the Adenoviridae family (formerly identified as group 1). There are several viruses included in this family, some considered primary pathogens while others considered secondary. Other notable poultry pathogens in this genus include Quail Bronchitis and Hydropericardium Syndrome. Siadenovirus (formerly Group 2) is responsible for Marbled Spleen Disease and Hemorrhagic Enteritis while Atadenovirus (formerly Group 3) causes Egg Drop Syndrome (1). The viruses are hardy and resistant to lipid solvents and changes in pH between 3 and 9. They are also able to survive temperatures up to 70°C (158°F) for up to 30 minutes (2). This resistance to elevated temperatures makes degradation in commercial poultry houses virtually impossible, even with windrowing. These viruses form basophilic intranuclear inclusion bodies making diagnosis by histopathology routine (Image 1). Gross lesions in livers are said to be of a "ground glass" or "sandy beach" appearance, and while not pathognomonic itself, are highly suspicious of the disease when coupled with age of onset and mortality pattern (Image 2). Polymerase chain reaction (PCR) targeting the hexon gene and virus isolation in cell culture are also used (3). Positive samples are further sequenced and those isolates may be saved for autogenous vaccine usage.



Image 1 (left). Intranuclear inclusion bodies (denoted by arrow) (40x mag) Image 2 (right). IBH gross lesion in liver

Fowl Adenoviruses (FAV) are classified into 5 species (A-E) based on whole genome sequences. Strains belonging to FAV group D and E can cause severe liver damage resulting in what we clinically call IBH (2). Mortality associated with IBH tends to occur for about 5 days following clinical presentation and can be quite sudden (2).

Mortality can range from minor to catastrophic with some cases in Australia reaching 30% (4). Incubation period is short at 24-48hrs with infection via natural routes and spreads quickly inside an affected house via the fecal oral route. There is no specific treatment for IBH but activated vitamin D and electrolytes in the water are typically used to minimize bone issues, such as rickets, later in the flock.

Since adenoviruses are quite hardy they can stick around in houses for a long time and create "repeater houses" where subsequent flocks also experience mortality associated with IBH. This can be frustrating for integrators as they try to combat this problem with limited tools at their disposal. The aim of this study was to learn to what extent IBH was an issue for integrators in the southeast US and the control measures that worked best for them.

Materials and Methods

In order to ascertain the impact of IBH on poultry companies and how it is being best controlled, a verbal questionnaire was used in which 5 companies in the southeast US responded. The following 7 questions were asked:

- 1) Have you had any IBH cases in the last year?
- 2) If so, what was the clinical presentation?
- 3) Did you have repeater houses?
- 4) What serotypes did you isolate?
- 5) Did you control IBH in your complex using autogenous vaccine?
- 6) Were there any other control measures used besides vaccine?
- 7) Is IBH currently an issue for you?

Data from submissions to UGA PDRC from 1-5-2021 to 1-4-2022 were compiled and positive cases (either by virus isolation and/or PCR with sequencing) were reported. Negative samples or positive samples where sequencing was not requested were not reported. These submissions included both domestic and international samples.

Results

Results from the integrator questionnaire are summarized below in Table 1 and UGA PDRC submissions were tabulated and listed below in Figure 1. All 5 companies that participated had at least one case of IBH in the last year. All 5 of the companies isolated serotype 8b while only 2 isolated serotype 11. This mirrors the UGA PDRC submission data with nearly 83% of positive cases being serotype 8b while serotype 11 was isolated just 12% of the time (out of 357 total cases). Age at onset of clinical IBH was divided into two distinct age groups. The first group was about 2.5 weeks old (16 days) and the other group was about 4 weeks old (26 days). Three companies saw mortality spikes at both time points while the other 2 companies only saw mortality in the older group. Four companies believed that their IBH cases were primary (the result of vertically shedding breeder flocks) while one company believed it was due to immunosuppressive diseases (primarily Infectious Bursal Disease). Remediation of IBH was split between the companies with 3 companies developing an autogenous vaccine and 2 companies relying on other intervention strategies.

Question	Company A	Company B	Company C	Company D	Company E
IBH in the last year?	Yes	Yes	Yes	Yes	Yes
Age at onset?	2.5wks and 4wks	2.5wks and 4wks	4wks	2.5wks and 4wks	4wks
Repeater houses?	yes	yes	no	yes	no
Serotype(s) isolated?	8b only	8b and 11	8b and 11	8b only	8b only
Autogenous vaccine used?	Yes	Yes	Yes	No	No
Other control measures?	Clean out and Disinfection	Clean out and Disinfection, heating houses	No	Clean out and Disinfection, windrow	IBDV control
IBH still an issue?	No	Yes	No	No	No

Table 1. Integrator questionnaire



Figure 1. UGA PDRC submission results

Discussion

The data from the UGA PDRC submissions and our questionnaire show that the predominant serotype detected in clinical IBH cases in the southeast US was largely FAV E/ FAdV 8b and FAV D/FAdV 11. These findings are similar to what was found in a recent 10-year retrospective study of broiler IBH cases in Spain where FAdV 8b was found 63.6% of the time and FAdV 11 was found 32.4% (5). The worldwide distribution of these serotypes, especially 8b, provides further evidence that this serotype is most likely a primary pathogen of chickens resulting in significant morbidity and mortality in affected flocks. It is important for integrators to maintain good surveillance for IBH in their operations and continue to gather isolates for possible autogenous vaccine usage to help minimize the impact of this disease in their operations. Autogenous vaccines take time to be manufactured and then time must pass until maternal antibodies are developed and reach the progeny being placed in the field. This study showed that other mitigation strategies, including removal of used litter, windrowing litter, and cleaning and disinfection of these houses can also be used effectively.

References

- 1) Fitzgerald S D. Adenovirus Infections. *In:* Diseases of Poultry, 14th edition, Swayne D E, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL., eds. Wiley-Blackwell, USA, pp 321-322. 2020
- 2) Hess M. Aviadenovirus Infections *In:* Diseases of Poultry, 14th edition, Swayne D E, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL., eds. Wiley-Blackwell, USA, pp 322-332. 2020
- 3) Benko M, Harrach B, and Russell WC. Family Adenoviridae. In Van Regenmortel M H V, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (eds.). Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press: New York and San Diego. pp 227–238. 2000
- 4) Barr D A and Scott P. Adenoviruses and IBH. Proc 2nd Asian/Pacific Poult Health Conference [Proc 112]. Post Graduate Comm Vet Sci, University of Sydney, Australia, pp 323–326. 1988
- 5) Bertran K, Blanco A, Antilles N, Nofrarías M, Valle RM, Cobos À, Ramis A, Biarnés M, Majó N. A 10-Year Retrospective Study of Inclusion Body Hepatitis in Meat-Type Chickens in Spain (2011–2021). Viruses. 13, 2170. <u>https://doi.org/10.3390/v13112170. 2021</u>



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