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▶ Q & A on Diagnostic Virology with Dr. Holly Sellers

Interviewed by Maggie Thompson

What services are available through the PDRC diagnostic virology lab?

We offer virus isolation, detection and characterization tests. Aside from the classical virology tests such as VI, titrations and virus neutralizations we offer many PCRs and real time PCRs to detect a majority of avian viruses.

What do you think the future holds for molecular diagnostics in the poultry industry?

There is definitely a shift in veterinary diagnostics towards next generation sequencing (NGS)/whole genome sequencing (WGS). This is an extremely powerful tool that would allow us to potentially identify all organisms in a sample. An abundance of sequence data is generated by NGS and requires a streamlined bioinformatics pipeline that allows for the assembly of the sequences for analysis. Full implementation of this technology in the avian diagnostic lab is hindered by a bottleneck at the bioinformatics stage. However, many are working in this area to determine the best use and implementation of this technology in the lab. One example of current use of NGS is for Newcastle disease viruses and Avian influenza viruses at the National Veterinary Services Laboratory/USDA, Ames, Iowa.

PDRC recently added a new diagnostic test for Infectious Bronchitis Virus (IBV) – a PCR panel which includes several serotypes and really changes the way we approach IBV diagnostics. Can you elaborate on this test & how it differs from the traditional diagnostic approach using virus isolation?

The IBV panel is a set of real time RT-PCRs (rRT-PCR) to detect IBV (positive/negative for all IBVs), IBV vaccines Mass, Ark, DE072/GA98, Conn and GA08 and several IBV variants that are or have caused disease in domestic flocks – DMV1639 and GA13. Results of this test are reported as cycle threshold (Ct) values and indicate the relative concentration of the IBV in your sample. The benefit of utilizing this panel is that you get a snapshot of all the IBVs in your sample. In the event that the sample contains an IBV not included in the panel (like a variant), the IBV+/- would be positive and the IBV specific rRT-PCRs would be negative. In this situation, virus isolation would be most appropriate to identify the IBV in the sample. The gold standard for avian respiratory viruses is virus isolation, but as many know, this can be time consuming taking up to 2 weeks to isolate a virus and then follow up with PCR and sequencing.

What samples should be collected & how should samples be submitted for this panel?

The best samples to submit for the panel are fresh tracheas (for acute respiratory disease) and cecal tonsils. In the case of nephropathogenic IBVs, including kidneys from affected birds is recommended.

What is the average turnaround time for the PCR test?

Results for the IBV panel vary depending on the laboratory caseload but should be within 3 business days.

Your team routinely performs diagnostics for Reovirus cases, specifically for potential autogenous vaccine production. What diagnostics are available for potential Reovirus cases?

The gold standard is virus isolation followed by Reovirus RT-PCR and sequencing for typing positive cases.

What are the best samples to submit for potential Reovirus cases and how should those samples be submitted?

Whole legs from affected birds are best samples for clinical cases of tenosynovitis. However, we rarely isolate viruses from birds with ruptured tendons, so those legs would not be the best samples to submit. For enteric disease cases, sections of the duodenal loop.

What is the average turnaround time for Reovirus diagnostics?

This varies – it can take up to 3 weeks to isolate the virus from a clinical sample since each passage takes about a week and for virus isolation, we make up to 3 passages of clinical samples. All reovirus isolates are confirmed by RT-PCR followed by sequencing and analysis.

In the past few years, have you observed any significant evolutionary changes with this virus?

Yes, extensive variation in the reovirus genome has occurred over time.

You also routinely perform diagnostics for monitoring Infectious Bursal Disease (IBD). Is it safe to say the more common presentation of IBDV in broilers is permanent immunosuppression associated with variant IBDVs?

Yes – we rarely have clinical IBDV cases submitted to the lab.

Are bursa surveys the best approach for an integrator to monitor for variant IBDV challenge?

Yes – bursal surveys have become a routine part of IBDV surveillance. The target age for collection of bursas is between 18-21 days of age. Collect bursas and put ½ of the bursa in 10% neutral buffered formalin and the other ½ in a whirlpak bag in the -80C freezer. Submit bursas for bursal scoring and do virus isolation on samples from farms with bursal scores of 3-5.

What is the most common variant your lab currently isolates?

AL2 has been the most prevalent variant isolated from bursal surveys for the past 4-5 years

In general, how should samples be shipped to the PDRC for virology diagnostics?

Ship samples as soon after collection as possible. Samples can be refrigerated overnight then shipped out the next day with expedited delivery in the morning. Ideal submission is fresh samples shipped on dry ice. If dry ice is not available, then ship on wet ice.

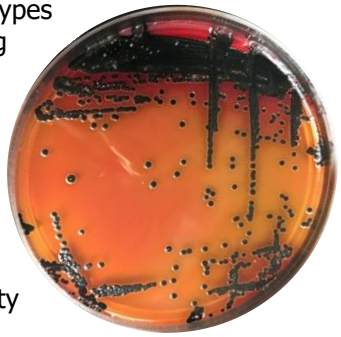
Samples can be refrigerated for up to 48 hours and then shipped on wet ice; however, this is risky during the summer and may result in skewed results or inability to perform any testing.

Dr. Holly Sellers is a professor and researcher at PDRC. She pursues clinical and molecular virology while also directing virology services and mentoring graduate and professional students at the PDRC. Very deservedly, Dr. Sellers was selected as the recipient of the 2019 UGA Inventor of the Year award which recognizes researchers for unique and innovative discoveries that contribute significantly to their designated industry. Notably, she was the first woman to receive this prestigious award since 2001.

New Technologies Offered at PDRC to Improve Food Safety

Dr. Nikki Shariat

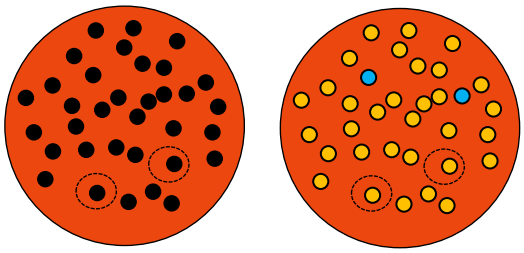
Developing an effective *Salmonella* control program hinges on knowing which serotypes are present. Ideally, the serotypes found in live production match those found during processing, as this helps direct serotype-specific strategies to reduce *Salmonella*, such as autogenous vaccination. However, this one-to-one pairing of serotypes between pre- and post-harvest is often not the case. Recent data from our research group at PDRC has shown that when *Salmonella* is identified in poultry, it often occurs in mixed populations of multiple serotypes, which can partially explain the discordance between live production and processing. Being able to identify multiple different serotypes in a single diagnostic sample helps our industry partners to understand the movement of *Salmonella* during broiler production to improve their food safety programs.



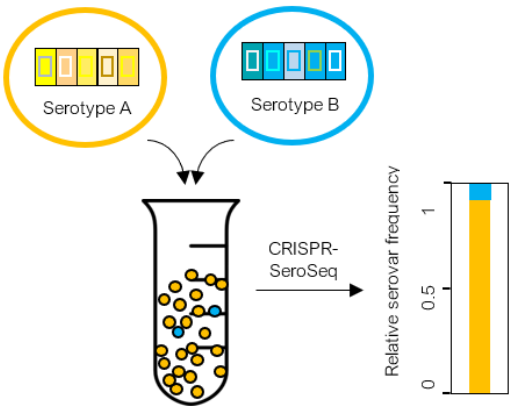
Salmonella is a leading cause of foodborne illness in the United States, and *Salmonella* mitigation remains a AAAP research priority.

The research and diagnostic focus of my lab is centered on understanding *Salmonella* serotype dynamics during food animal production. One of the biggest problems faced is that during *Salmonella* isolation, only a small number of colonies are picked off a plate and characterized. When multiple serotypes are present, this approach results in only the most abundant serotypes being isolated (see "problem" below).

Problem: during routine surveillance, 1-2 colonies (dotted circles; left) are isolated and characterized, typically to identify the serogroup or serotype. Where there are multiple serotypes found in a sample, routine surveillance approaches therefore favor the serotypes that are most abundant (yellow; right) and those less abundant are often not captured (blue; right).



We have developed a diagnostic tool called, CRISPR-SeroSeq, that allows us to easily identify multiple serotypes in an individual sample (see "solution" below) by exploiting the serotype-specific CRISPR sequences found in *Salmonella*. These short snippets of DNA occur naturally in *Salmonella* and can be thought of as barcodes, where each barcode is associated with a single serotype. CRISPR-SeroSeq dovetails nicely with existing protocols for *Salmonella* isolation: the "input" for this method is a small aliquot of an overnight selective enrichment broth (e.g. tetrathionate culture). Total DNA is isolated from this culture and then a PCR is performed that targets the CRISPR sequences. After sequencing of the CRISPR products, we use bioinformatics to match the sample CRISPR sequences to those in our database of 135 known *Salmonella* serotypes. Going back to the problem posed above, this deeper information can now help us address whether serotypes match between pre- and post-harvest.



Solution: CRISPR-SeroSeq uses the serotype-specific CRISPR barcodes (top; colored boxes) to be able to identify different serovars present in a mixed population (middle). This method uses PCR and sequencing to reveal the relative abundance of each serovar in a mixed population (right).

There is an important distinction between CRISPR-SeroSeq and whole genome sequencing: the latter identifies every single DNA base and is used for source tracking during *Salmonella* outbreaks, where strain-specific information is key. CRISPR-SeroSeq uses a PCR step that targets just the CRISPR region, and these PCR products are then sequenced to identify the serotype.

Thus far, our lab has used CRISPR-SeroSeq to examine *Salmonella* serotype populations in a variety of different sample types including live production (bootsocks, drag swabs, feed, feed mill dust) and processing (carcasses pre- and post-chill, parts). We are now offering CRISPR-SeroSeq as a diagnostic at PDRC – please see our catalog for more information.

Dr. Nikki Shariat has been working in food safety for over a decade and is an expert in developing molecular methodologies for analyzing Salmonella. Dr. Shariat joined the faculty at PDRC in March 2019 as an assistant professor and her research group is centered on understanding Salmonella serotype populations in food animal production and in the environment.

▶ Useful Links:

PDRC Diagnostic Services Homepage

PDRC Diagnostic Lab Test & Fee Catalog

PDRC Diagnostic Lab - Domestic Submission Form

▶ Interesting Topics at AAAP 2021

Reviewed by the MAM Students

Reoviral Hepatitis in Commercial Hen Turkeys – Dr. Laura Tensa

This case study presented by Dr. Laura Tensa involved the investigation of reoviral hepatitis in three different farms that had a source flock in common. Everything began when the flock supervisor reported ~0.5% mortality in 10-day poults in only one barn (flock A) on the affected farm. The flock supervisor reported that overall the flock was active, and the mortality was on one end of the barn. Necropsy findings included pinpoint white hepatic foci on livers and hepatomegaly. Liver samples were sent to the diagnostic lab and the flock was not treated. A few days later in flock B, there was ~0.5% increased mortality in 10 days old poults. The necropsy findings were similar to flock A and liver samples were sent for diagnosis and the flock was not treated. Also, Flock C had increased mortality and similar clinical signs as flock A and B, no treatment was elected, and frozen liver samples were sent for analysis. Lab diagnostics show no aerobic growth in 48 hours in flock A, histopathology in flock A and B showed lesions consistent with reoviral hepatitis and in all flocks, reovirus was isolated. Interestingly, all the flocks shared a common source flock that was in mid-lay. There were no changes in egg quality or production, and there had been no complaints with poult quality Titers increased from 546 at 32 weeks to 11401 at 44 weeks, which shows that this flock was challenged with reovirus. On follow up visits to affected commercial flocks, affected birds were stunted, the flocks were uneven, and birds had swelling below the hock with ruptured tendons. At the processing plant, many of the birds have fresh bruising and ruptured leg tendons leading to downgrades and increased condemns. Flock comparison to a three-week average, there was decreased livability, increased condemns, decreased weight gain, increased feed conversion, and decreased gain/day. There was an increase in medication usage compared to non-affected flocks where approximately 73.75% in scripts were respiratory in nature, and of those, 38.6% had treatment failure, which was represented by increased duration or switch of drug. Tenosynovitis arthritis was seen in commercial flocks ranging from 0.8% to 100% placements out of the shedding breeder flock. There was horizontal transmission between birds and vertical transmission from the source flock; however, no tenosynovitis arthritis was seen in adjacent barns that did not contain poults from the infected breeder flock, even though poults were placed in the same day. To prevent Reoviral Hepatitis, all brood barns were cleaned and disinfected before placing new chicks; therefore, they did not see hepatitis or ruptured tendon in subsequent flocks placed on the farm.

Summary Provided by: Roel Becerra

Pickled pullets? - Dr. Jenny Nicholds

This case report presented an investigation into neurological signs and increased mortality in 12-day old pullets. A portion of birds with neurologic signs, such as ataxia, eventually recovered suggesting a toxicity or metabolic disturbance. Initial diagnostic tests included post-mortem exam, blood glucose measurement and histopathology. Histopathology demonstrated Purkinje cell necrosis in the cerebellum which is associated with zoalene toxicity. A farm visit followed where affected birds and feed samples were collected for further analysis, at this point cumulative mortality had reached 2.48%. Dr. Nicholds recalled a case previously presented at AAAP by Dr.Kabel Robbins and suspected imidacloprid toxicity. Dominion, a pesticide containing imidacloprid, had been used at this farm. To investigate this further samples of litter and crop contents were sent to the Michigan State University Veterinary Diagnostic Lab for analysis. Both litter and crop samples tested positive for the presence of imidacloprid. It is theorised that an application error led to imidacloprid contaminated litter and as pullets became increasingly feed restricted litter consumption increased resulting in presentation of imidacloprid toxicity. Histomoniasis and intussusceptions were also identified in this flock. Excess coccidial cycling with E. tenella species likely precipitated the Histomoniasis disease presentation, and was exacerbated skip a day feeding. In response to intussusception and mortality fluctuating with skip a day feeding the birds were switched to everyday feeding. The intent was to help manage intestinal motility and to reduce the quantity of litter consumed, therefore reducing the incidence of intussusception and imidacloprid toxicity respectively. The liver damage caused by Histomoniasis likely increased the incidence of imidacloprid toxicity as this compound is

hepatically metabolized. Several red herrings presented themselves in this case including the presence of wild birds near the farm and consumption of beetles which has been associated with botulism. Luckily, the correct diagnosis was reached through logical thinking and appropriate diagnostic tests!
Summary Provided By: Isabella Hannay

Botulism in Broiler Breeders: Dr. Kurt Dobson & Dr. Sara Throne
This year, two case reports on *Clostridium botulinum* neurotoxicity in broiler breeders were presented by Dr. Sara Throne (Simmons Foods) and Dr. Kurt Dobson (George's).
The clinical presentations were quite similar in both cases. Dr. Dobson's cases were observed over 5 consecutive flocks, all originating from the same house on a single pullet farm. An interesting pattern was observed; the first case occurred once birds were moved to the hen farm, the second case occurred on the pullet farm, third case on the hen farm, etc. which seemed to be associated with whether pullets were placed on built up litter or not. Dr. Throne's case involved a single house on a 4-house hen farm. In both cases, peak mortality on the hen farm correlated to onset of production between approximately 25 and 27 weeks of age. Affected birds were lethargic, reluctant to move, had occasional weepy vents and were exhibiting the classic "limberneck" posture which, thanks to the NAVLE, we all remember to be a tell-tale sign of *C. botulinum* infection. Due diagnostic diligence was performed for both cases and Botulism Neurotoxin Assay (Mouse Bioassay) confirmed infection with *Clostridium botulinum* Type C neurotoxin. No source of infection was ever identified in either case, even though many possibilities were investigated including feed and water contamination and other environmental sources. All cases responded well to treatment with either penicillin or tylosin and once clinical signs resolved, mortality and production returned to normal. Personally, these cases served as a reminder that *Clostridium spp*. is like a box of chocolates for chickens – you never know what you'll get.

Congratulations to Dr. Throne for winning the 2021 AAAP Outstanding Field Case Report Award for her presentation titled, "Sunday Morning Calls Are Never Good"!
Summary Provided By: Maggie Thompson

▶ Meet the 2022 MAM Candidates

Maggie Thompson

Maggie was born and raised in Opp, Alabama, home of the famous Rattlesnake Rodeo. She all but stumbled into the poultry industry while seeking alternatives to veterinary school (because backup plans are important). During an undergraduate summer internship, she spent time with Dr. Joel Cline and it was through that experience she realized a facet of veterinary medicine existed for her to combine her newfound appreciation for the poultry industry and her interest in veterinary medicine. She earned both a Bachelor of Science in Poultry Science (2013) and a Doctor of Veterinary Medicine (2018) degree from Auburn University. She then spent three years working with Elanco as a poultry technical consultant covering areas of Tennessee, Alabama, Mississippi, and Georgia. Understanding the importance of both knowledge and experience, she returned to school to strengthen her skills as a poultry veterinarian. While she is no fan of rattlesnakes, she remains proud to call south Alabama home. So, when she isn't in Athens, she can be found in the beautiful Wiregrass region. She and her husband Andrew are proud parents of a little boy, Amos, and four rescue dogs.

Isabella Hannay

Isabella grew up on several sheep farms across England and Ireland. Her dream to become a farm animal veterinarian started early with a love for lambing and milking. She discovered poultry medicine after starting veterinary school at the University of Nottingham. The fast-paced and forward-thinking aspect of the poultry industry meant chickens became her focus. Many placements and research projects with poultry veterinarians followed where she gained experiences with not only layers and broilers, but also Humboldt penguins, commercial pigeons, and partridges. During vet school, Isabella completed a research masters which studied the effect of dietary enzymes on broiler growth rates. Isabella was slated to begin the MAM program in June of 2020 however, the COVID-19 pandemic had other plans. Instead, she spent a year working for Aviagen in Scotland which was a great opportunity to learn about primary breeders and spend time in hatcheries. This year, she finally made it across the pond to start the MAM. She feels extremely fortunate and is determined to make the most of this opportunity. Outside of poultry, Isabella enjoys spending time with her rescue dog, Skippy, and enjoying the outdoors with friends.

Roel Becerra

Roel was born in a small town located in Zacatecas, Mexico. He earned a Doctor of Veterinary Medicine degree from Purdue University in 2021. He has over 8 years of experience in food animal production and has chosen to focus on poultry. He is fluent in English and Spanish, and enjoys working with individuals of different cultures and ethnicities. His motivation for pursuing a Master of Avian Medicine is two-fold: a strong desire to become a poultry veterinarian, and an interest in research to innovate and improve protocols in poultry medicine and production. His dream to become a poultry veterinarian began as a child in his home country of Mexico as he worked on the family poultry, beef, and sheep farm. When the animals became ill, there was a high probability they would not survive since there were no veterinarians in his hometown. As a young child, he recalls losing all his backyard chickens to Avian Influenza. Not knowing how to attend to or maintain their health was a great source of frustration. Veterinarians were located too far away, and their fees were often more than the value of the animals. These events initiated his desire to become a vet, to fill the void by providing quality medicine for lower income communities. Through his coursework, practical experience and research opportunities at Michigan State University, North Carolina State University, and Purdue University, his knowledge in poultry science has increased greatly. This path has led him to continue his studies in the MAM program to specialize in avian medicine and support his desire to work with poultry. His life's work is to better understand poultry needs and address the urgency to improve the health and welfare of poultry species.