New Equine Tests and Resources
by Lisa Tadros, DVM, PhD, DACVIM

To better support clients using our laboratory for their equine patients, we have created new orderables specific to equine endocrine testing, a new equine-specific submittal form, and two new client education guides to help clinicians provide information to horse owners. All of these are available on our website. The following tests have been added to our online test catalog which contains specimen, collection, shipping requirements, and other information:

- Thyrotropin-Releasing Hormone (TRH) Stimulation Test (20066)
- Overnight Dexamethasone Suppression Test (20254)
- Equine Oral Sugar Test (20552)

These new tests support clinicians in diagnosing Pituitary pars intermedia dysfunction (PPID) and Equine Metabolic Syndrome (EMS) while the client education guides assist in explaining these conditions to owners.

PPID occurs when oxidative stress reduces tonic dopaminergic inhibition of pars intermedia melanotropes, giving rise to hyperplasia, microadenomas, and macroadenomas. Detecting excessive endogenous plasma adrenocorticotropic hormone (ACTH) derived from the abnormal pars intermedia is the most common diagnostic test for PPID. Although comparable in performance to endogenous ACTH, the overnight dexamethasone suppression test (ODST) is more laborious and is therefore falling out of favor. Sensitivities of both endogenous ACTH and the ODST are acceptably high in equids with advanced PPID, but not in mild cases. A more sensitive option is the thyrotropin-releasing hormone (TRH) stimulation test (measuring ACTH response). Insulin dysregulation is common and is likely associated with an increased risk of laminitis and poorer long-term prognosis; testing for insulin dysregulation is recommended in all PPID cases.

Key Points

- Endogenous ACTH has supplanted endogenous cortisol measurement.
- All PPID tests may yield false positives in animals with severe systemic illness, stress, or pain.
- Rather than avoiding autumn (mid-July to mid-November) testing, exploit heightened seasonal responsiveness of the hypothalamic-pituitary-adrenal axis to increase diagnostic test sensitivity. Currently, seasonal reference ranges are only established for endogenous ACTH.

Endogenous ACTH concentration:

- Collect EDTA plasma at any time of day
- PPID is supported by ACTH concentration > 10 pmol/L (45 pg/mL)
- From mid-July to mid-November: > 22 pmol/L (100 pg/mL) supports PPID

Overnight dexamethasone suppression test:

- Collect baseline serum cortisol sample in the late afternoon
- Administer dexamethasone at 40 µg/kg IM (20 mg to a 500 kg horse)
- Collect serum cortisol sample(s) between 15 – 19 hours later
- Failure of cortisol suppression < 30 nmol/L supports PPID

Thyrotropin-releasing hormone stimulation test:

- Do not perform immediately after an oral sugar test due to blunting of pituitary ACTH responses to TRH
- Collect baseline EDTA plasma sample for ACTH measurement
- Administer 1.0 mg (total dose) of TRH IV
- Collect EDTA plasma ACTH sample exactly 10 minutes after TRH administration
- An ACTH concentration > 25 pmol/L (110 pg/mL) supports PPID
Low Pathogenic Avian Influenza (LPAI) H5 Virus Detections in Michigan Wild Birds

by Annabel G. Wise, DVM, PhD and Richard M. Fulton, DVM, PhD, Dipl. AcPV

In late June 2015, the MSU Diagnostic Center for Population and Animal Health (DCPAH) was one of seven National Animal Health Laboratory Network (NAHLN) laboratories invited by the USDA Animal and Plant Health Inspection Service (APHIS) Wildlife Services to participate in a nationwide avian influenza surveillance of wild birds. This large-scale surveillance, targeted testing of approximately 45,000 wild waterfowl, was in response to the devastating outbreaks of highly pathogenic avian influenza (HPAI) H5 in domestic poultry that affected up to 15 states from December 2014 to June 2015 and resulted in the loss of nearly 50 million birds. The HPAI H5 virus was also detected in wild birds from 6 states, including Michigan where sick Canada geese collected from Macomb County tested positive for HPAI H5N2 in June 2015.

From July 2015 to March 2016, DCPAH tested a total of approximately 5,500 wild bird samples submitted by the USDA and state wildlife agencies from seven states, including Michigan. Of local importance, the Michigan Department of Natural Resources (MDNR), in collaboration with USDA Wildlife Services in Michigan, collected and submitted approximately 850 wild waterfowl samples from 49 counties in Michigan during the 9-month surveillance period. The samples were taken mostly from hunter-harvested birds, with a few from morbidity and mortality cases. No HPAI H5 was detected; however, there were positive detections of low pathogenic avian influenza (LPAI) H5 in wild bird samples, mostly from mallard ducks, collected from 10 of the 49 counties sampled (see Figure). Non-H5/H7 AI virus detections were made in four other counties. All LPAI H5 detections were confirmed by the National Veterinary Services Laboratories in Ames, Iowa.

The finding of LPAI H5 in wild waterfowl in 10 counties in Michigan is significant, but not unexpected because these viruses naturally occur in some wild waterfowl. LPAI H5 has been diagnosed previously in a Michigan commercial poultry flock, as well as a commercially-raised release for hunting mallard flock. Although H5/H7 or non-H5/
H7 AI viruses were not found in other counties, it does not mean that the waterfowl in those counties are not able to harbor those viruses. Wild waterfowl are recognized to be the natural reservoir host for avian influenza viruses. LPAI infections in these birds are generally asymptomatic with the virus remaining genetically stable as it cycles through the wild population. LPAI viruses, upon transmission to highly susceptible poultry species, such as chickens and turkeys, only cause mild clinical signs or no apparent disease at all.

However, as these LPAI viruses cycle through these poultry species, the H5 and H7 subtypes in particular have the propensity to undergo a series of mutation events that lead to host adaptation and a switch from low to high pathogenicity. This genetic switch is marked by the acquisition of a polybasic cleavage site in the hemagglutinin (HA) gene that then gives the HPAI virus unrestricted ability to replicate throughout the host and cause devastating disease. In January of this year, a HPAI H7N8 outbreak occurred in a poultry farm in Dubois County, Indiana where there were simultaneous detections of the LPAI H7N8 on 8 different farms in a concentrated poultry area. Genetic analyses of the strains led to the conclusion that the HPAI H7N8 strain arose from its low pathogenic precursor as it circulated from farm to farm.

The chance of wild birds transmitting avian influenza infection to domestic poultry is highest among domestic birds reared in the open and close to bodies of water where wild birds may reside. This “free-range” set-up allows for domestic flocks to be exposed to contaminated water and/or food sources from the fecal droppings and oral secretions of flu virus-carrying wild waterfowl. In this situation, unless the flock is subjected to periodic testing for the presence of virus infection, the more often than not “silent nature” of LPAI infection in these birds will remain undetected until disease becomes apparent. In any size of poultry holding, isolation of the flock with man-made barriers, along with consistent observance of appropriate biosecurity practices, is key to preventing the introduction of the virus from the wild into the farm. Producers may contact the USDA APHIS Wildlife Services’ state office at 1-866-4USDA-WS (1-866-487-3297) for concerns regarding wildlife issues involving their facilities. For more information on avian influenza and recommended biosecurity practices, please visit the USDA website at www.usda.gov/avianinfluenza.

References:
AI SITUATION UPDATE 2/18/16 State of Indiana Response to Avian Flu in Dubois County 2/18/16: http://www.in.gov/boah/2759.htm
Editor’s Note: A version of this article was also published in the June 2016 issue of Michigan Veterinarian.
A Message from the Director

Greetings all, from the faculty and staff of DCPAH. After a false start, spring appears to finally be here! The energy and enthusiasm of spring are evident in the new graduates as they prepare to launch their new careers. To the class of 2016: Welcome new colleagues!

This quarter has been one of the busiest for DCPAH since 2009. In addition to increases in submissions from referring veterinarians, the laboratory tested approximately 5,500 wild bird samples as part of the surveillance program for Avian Influenza (read more about that on pages 2-3). With the discovery of Chronic Wasting Disease in wild deer in the state, the laboratory has tested a record number of samples (more than 7,500) while working in close partnership with the Michigan Department of Natural Resources. DCPAH is actively recruiting for two new scientists for the Endocrinology section, a scientist in Immunodiagnostics/Parasitology, as well as two anatomic pathologists to meet the demand in these growing and busy sections. We look forward to having new diagnosticians on the DCPAH team.

Since our last newsletter, the laboratory has added six new offerings to our test menu. I would like to highlight one of these offerings. A new PCR assay for Seneca Valley Virus (SVV) was developed in October as the first cases of this disease were diagnosed in Michigan. The clinical signs of SVV and Foot and Mouth Disease, a devastating disease in multiple species, are indistinguishable. As required by USDA, these cases were treated as Foreign Animal Disease (FAD) suspects. Given the potential impact should FMD or another FAD occur in the state, time is critical. Therefore, while these cases were sent to the National Veterinary Services Laboratory for FAD investigation, they were also simultaneously screened by DCPAH leveraging our Biosafety-level 3 laboratory. Working closely with MDARD, we were able to provide same day test information on multiple submissions throughout fall 2015. While we were already able to perform FMD testing, we did add FMD to our test catalog along with SVV. It’s worth noting that neither of these tests is visible to clients on our website because they are ordered in conjunction with an FAD investigation through state or federal animal health officials and would not be ordered independently by a practitioner.

The staff and faculty of DCPAH have also been actively engaged in the strategic planning activities of the College and the laboratory. Part of that planning involved important feedback from our clients concerning the need for more continuing education opportunities. In response, DCPAH is planning a series of webinars throughout 2016, and a half-day CE workshop in the fall. See page 3 for more information on what we have in store this year.

Well wishes during this time of spring weather and activities.

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Rachel Riems