

DIAGNOSTIC CONNECTIONS

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Local. Trusted. Proven.



**Florida Department of
Agriculture and Consumer Services**

FROM THE DIRECTOR

Yugendar “Reddy” Bommineni, DVM, PhD, DACVM, DACPV

Since April 8, 2020, the Bronson Animal Disease Diagnostic Laboratory (BADDL) has enacted a mission critical plan to reduce staffing at the laboratory during the Florida Safer at Home COVID-19, Executive Order Number 20-91. The laboratory will continue to receive and process limited samples from our clients. Clients dropping off samples should fill out paperwork and place the samples in the drop-off coolers in front of the Receiving doors. The goal is to continue to provide mission-critical services to animal industries of Florida. Turnaround times could vary depending on availability of staff, but reasonable efforts will be made to provide quality results in a timely manner. BADDL will classify samples and necropsies into priority groups and they will be processed accordingly.



BADDL COVID-19 Operating Services Update



Priority 1 - Samples will be processed immediately:

- Foreign Animal Diseases (FADs), National Animal Health Laboratory Network (NAHLN) diseases, and Florida reportable diseases.
- Any samples from clinically ill, live animals submitted by veterinarians (swabs, exudate, urine, or biopsies).
- Food and fiber animals and poultry species will be tested first within this category.
- Forensic cases from governmental sources such as county animal services, police departments, or sheriff's offices.

Priority 2 - Samples will be processed when staffing allows:

- For samples that do not meet the above criteria (Priority 1), submitters will be given information of other diagnostic laboratories that may be accepting samples to avoid potential delays in testing.
- If submitter agrees to delayed testing of Priority 2 samples, the submitted samples will be stored in a refrigerator and tested as soon as staffing allows.

Necropsy Considerations:

Submitters are encouraged to call the laboratory prior to delivering necropsy submissions.

Based on the best scientific judgement of the pathologist on the floor, a gross-only necropsy will be performed on each case. Food animal species, FAD, Reportable and Zoonotic disease cases will be given priority. The specific daily prioritization will be determined by the pathologist on duty and/or Laboratory Director. BADDL may not accept other forensic, research, or insurance-related necropsies of companion animals and horses. This decision will depend upon availability of staff and the necropsy cooler space. BADDL will only accept companion animal necropsies when there are potential contagious/infectious agents involved (Rabies, *Salmonella*, *Campylobacter*, etc.).

We appreciate your patience and thoughtfulness as we are limiting our staffing and interactions during the COVID-19 response. If you have any questions, please do not hesitate to call our BADDL team at 321-697-1400.

National Animal Health Laboratory Network (NAHLN)

Mock Exercise Participation – ASF Oral Fluids

On September 15-16, 2020, BADDL participated in a NAHLN Emergency Validation Exercise. Participating in these types of exercise scenarios give representatives from the NAHLN laboratories, NAHLN program office, and reference laboratories the opportunity to practice the Emergency Validation Processes. The validation processes are used to validate a new sample type after a foreign animal disease detection/outbreak. This exercise focused on the Emergency Validation Process and did not include testing of samples. The main objectives were:

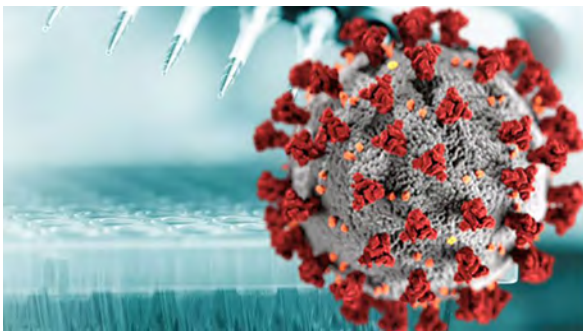
- For participating labs to exercise the steps of the Emergency Validation Process.
- Confirm the testing and data collection protocols which will be used during the Emergency Validation Process by analysts who have successfully completed a proficiency test.

The scenario for this exercise assumed that African Swine Fever (ASF) had been confirmed within Florida by NVSL and a Unified Incident Command had been established. ASF is an emerging disease that is currently threatening the swine population throughout the world. It is caused by a large DNA virus of the Asfarviridae family. It can be transmitted by direct contact with infected pigs, by the bite of a tick, or by indirect contact with contaminated materials or fomites. ASF is a highly contagious viral hemorrhagic disease of domestic and wild pigs which can cause serious economic and production losses.

Currently, oral fluids are not an approved sample type to test ASF. This Emergency Validation Exercise allowed the lab to practice the steps necessary to seek approval to deviate from normal testing protocols. This process involved the Laboratory Director, Emergency Laboratory Coordinator Liaison, Microbiology Department Head, and Molecular Diagnostics Section Head and Section. The activities BADDL participated in included:

- Submitting a deviation to test the unapproved sample type.
- Participating on a conference call with the Management Team Working Group (MTWG) Emergency Approval Incident Management Team to plan testing, discuss field sampling logistics, the protocol that should be used, and reporting expectations.
- Reporting results on a spreadsheet to the NAHLN program office.
- Participating in a hotwash after the completion of the exercise.





Bronson Animal Disease Diagnostic Laboratory (BADDL) Approved to Test Animals for SARS-CoV-2

A collaborative group including the Florida Department of Health (FDOH), the Florida Department of Agriculture and Consumer Services (FDACS), the University of Florida, College of Veterinary Medicine (UF CVM), and the Florida Fish and Wildlife Conservation Commission (FWC) have been approved to start testing animals for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) through a grant to better understand the role of animal transmission in SARS-CoV-2. Targeted animal groups identified in event-based surveillance will be tested for the presence of SARS-CoV-2. The BADDL will perform PCR tests in cats, dogs, and any other species previously found to be susceptible to SARS-CoV-2 infection. Serology samples will be submitted 4-6 weeks after the event to determine transmission within these collections.

The following criteria, based on the Centers for Diseases Control and Prevention Guidelines, will be followed to collect samples for laboratory testing for SARS-CoV-2 in Animals:

1. Animals that have been evaluated by a veterinarian.
2. An animal with a history of close contact with a person or animal confirmed to be infected with SARS-CoV-2.
3. An animal with exposure to a known high-risk environment (i.e., where human cases or animal cases have occurred) such as a residence, facility, or vessel (e.g., nursing home, prison, or cruise ship).
4. A threatened, endangered or otherwise imperiled/rare animal in rehabilitation or a zoological facility with possible exposure to SARS-CoV-2 through an infected person or animal.
5. Animals in a mass care or group setting (e.g., farm, animal feeding operation, animal shelter, boarding facility, zoo, or another animal holding facility) including companion animals, and other species, where their exposure history to people with COVID-19 is unknown.
6. Wildlife with possible exposure to SARS-CoV-2 as determined by FWC, FDOH, and FDACS.

If these conditions are met, please contact the State Veterinarian's office at (850) 410-0900 or RAD@FDACS.gov, or the Florida Public Health Veterinarian's Office at (850) 245-4117 or (850) 245-4401 to receive authorization. Authorized testing will be performed by the State without cost to the owner. In order for the animal testing to occur, the owner must complete a confidential history form and agree to both a nasal swab and a blood test for the animal. In addition, the owner must agree to provide serum for a blood test at 3-6 weeks after initial testing.



PATHOLOGY CORNER

The Case of a Neurologic Arabian Colt

Performed by: Joanna Hyland, DVM, MS, Diplomate ACVP

Case Signalment & History:

A colt was found down and tangled in the tree branches and fencing in the morning. He was normal the day prior. He was recently relocated to the property three days prior from the same subdivision. The colt did not have a fever and had a toxic line on his gums. Neurological signs were localized to the forebrain/cerebrum (centrally blind, no response to noxious stimuli, intact pupillary light reflex (PLR)). There were no obvious abnormalities on lateral skull or neck X-rays as reported by the initial examining veterinarian. The complete blood count (CBC) showed a stress leukogram (neutrophilia, lymphopenia, monocytosis, and eosinopenia) and normal bile acids. There were no abnormalities on blood chemistry. The colt had become progressively manic and dangerous, even after treatment with 35 mg/diazepam IV. Vaccinations included: Eastern Equine Encephalomyelitis (EEE), West Nile Virus (WNV), Tetanus, Influenza, Equine Herpes Virus (EHV)-1 and EHV-4. The colt was treated with intravenous fluids, flunixin meglumine, Dimethyl Sulfoxide (DMSO), and dexamethasone. Differentials included viral (Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE), West Nile Virus (WNV), Rabies), bacterial, fungal, parasitic/protozoal (EPM, *Halicephalobus*), hyperammonia encephalopathy (liver or GI in origin or a portosystemic shunt), a mass (abscess/tumor), or toxicity.

Necropsy Findings:

The body of a 14-month-old, male, Arabian was submitted for standard necropsy. The horse was in good body condition with adequate subcutaneous and visceral adipose tissue. Autolysis was mild. There was a moderately increased amount of thin, clear, watery cerebrospinal fluid that flows from the spinal canal. Within the tracheal lumen there was a moderate amount of clear, yellow, foamy fluid. The pericardial sac contained a small amount of thin, blood-tinged fluid. Within the peritoneum was a small amount of clear, yellow fluid. The spleen was diffuse, mildly enlarged and blood flowed from cut surfaces. All other tissues examined were grossly within normal limits.

Brain: edema, diffuse, moderate, acute.

Microscopic Examination:

Cytology of Cerebrospinal fluid (CSF) - 10 ml of clear fluid was processed and revealed a few well-differentiated lymphocytes over a slightly proteinaceous background with no other cellular elements noted.

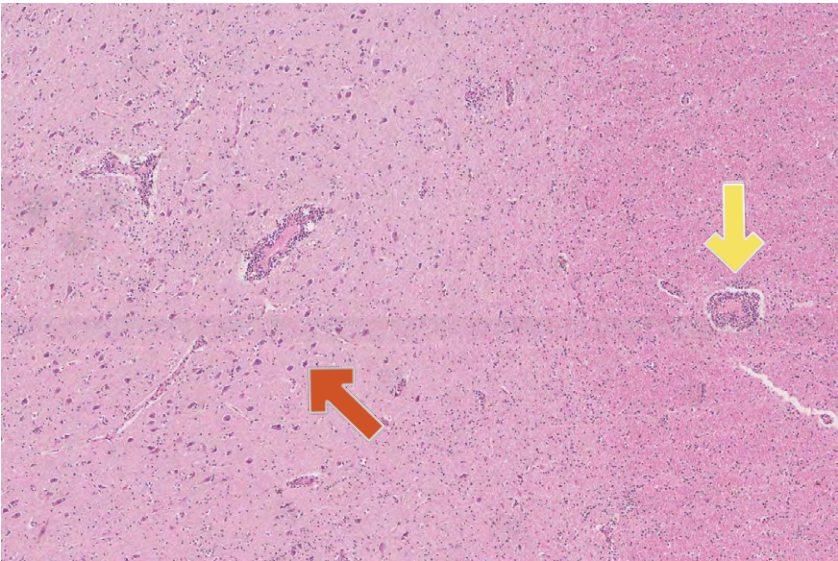


Fig 1. Diseased Brain. Meningoencephalitis. Expansion of the perivascular spaces (yellow arrow) by edema, hemorrhage, necrotic cellular debris, and an inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, and macrophages. There is diffuse gliosis, including microgliosis. Scattered neurons are necrotic with shrunken, angular cell bodies, increased cytoplasmic eosinophilia, and pyknosis (orange arrow). Hematoxylin & Eosin (H&E).



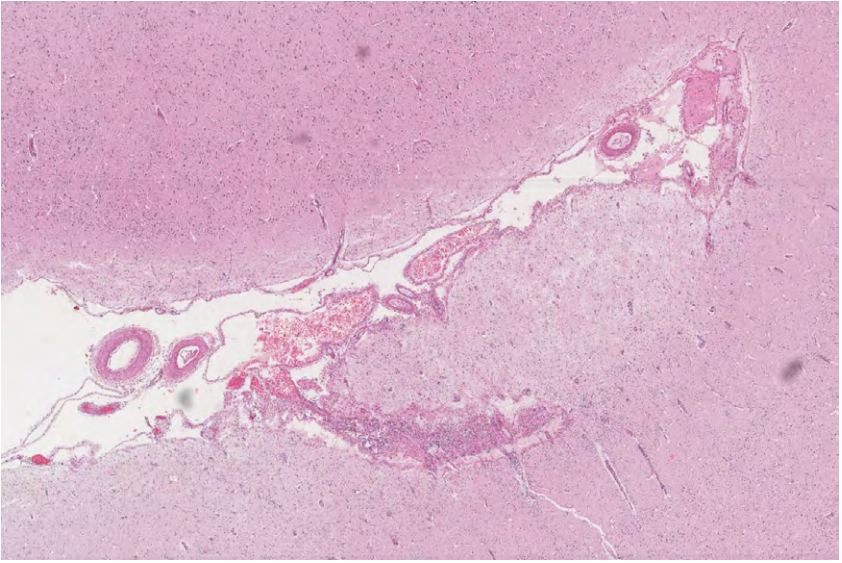


Fig 2. Diseased Brain. Meningoencephalitis. Multifocal to confluent, moderate to marked expansion of the meninges and perivascular spaces by edema, hemorrhage, necrotic cellular debris, and an inflammatory infiltrate. H&E.

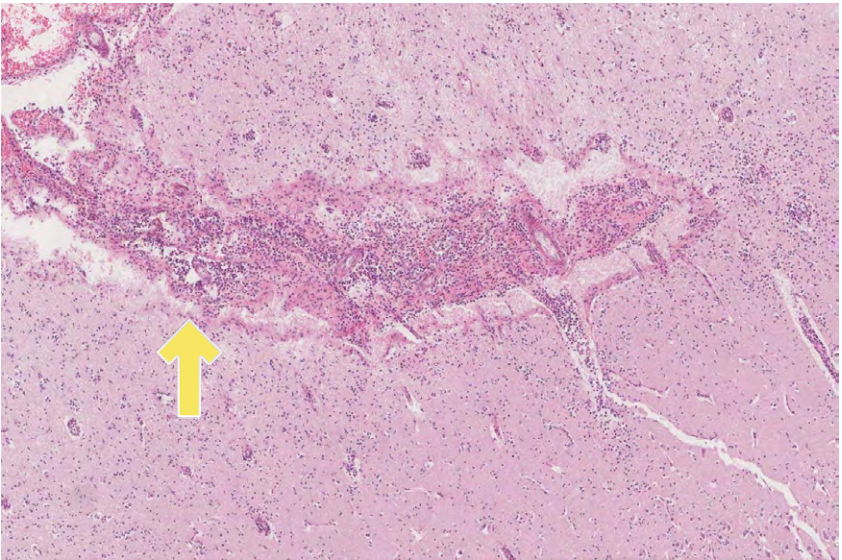


Fig 3. Diseased Brain. Meningoencephalitis. There is multifocal to confluent, moderate to marked expansion of the meninges (yellow arrow) by edema, hemorrhage, necrotic cellular debris, and an inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, and macrophages. The inflammatory cells frequently extend into the adjacent parenchyma with rarefaction of the affected neuropil. H&E.

With the exception of congestion, multiple tissues including lung, heart, liver, spleen, kidney, gastrointestinal tract, thymus, thyroid gland, and testicle are microscopically within normal limits.

Morphological Diagnoses:

Brain: neutrophilic and lymphohistiocytic meningoencephalitis, multifocal to confluent, marked, subacute.

Finding from ancillary testing included:

Virology:

Rabies FA performed on the brain was negative.

Molecular Diagnostics:

Samples of fresh brain were positive for EEE Virus via PCR. Samples of fresh brain were negative for WNV and St. Louis Encephalitis Virus via PCR. Equine Herpes Virus 1 and 4 were not detected.

Bacteriology/Mycology Examination:

No growth cultured from the spinal cord fluid. Light growth of *E. coli* was isolated from urine.

Parasitology Examination:

Fecal specimen revealed severe numbers of Strongyle-like parasite ova.

FINAL DIAGNOSES:

The PCR was positive for EEE and microscopic findings of meningoencephalitis are consistent with EEE infection. EEE is an arbovirus and a zoonotic disease that can cause fatal disease in humans, horses, and other mammalian and reptilian species. Arboviruses are viruses that are transmitted by mosquitos or other blood-feeding insects. Birds and rodents can be the reservoirs for transmission, with humans and horses becoming infected during the transmission cycle via bites from infected mosquitos. Young horses are more susceptible than old, and infections occur typically in the late summer and fall seasons. Horses and people do not develop viremia (virus levels in the blood) sufficiently high enough to transmit virus to mosquitoes, which is why they are called “dead-end” hosts. The target cell in the CNS is the neuron. There is no specific treatment for viral encephalitis. The primary method of prevention is adequate vaccination. Deaths occur within two to three days after signs appear. Human cases are rare, with an average of eight cases nationwide annually over the past few years. The virus is highly regulated as it has been cited as being considered a potential biological weapon capable of aerosolization.



Tests available at BADDL

		Fresh tissues or body fluids in a sterile, leakproof container or syringe; shipped chilled within 24 hours of collection. Swabs in aerobic transport media; shipped chilled overnight within 24 hours of collection.	
Biopsy	\$40 first tissue specimen per animal \$10 each subsequent tissue specimen	Fixed tissues in a leakproof container with a screw-on lid (should be put in a Ziploc bag with paper towels/absorbent material).	5 days
Cytology	\$30.00 per site	Air-dried or fixed slides and/or fluid (preferably preserved in EDTA tube) submitted in a plastic or cardboard slide container or leakproof tube for fluid samples. Unpreserved fluid should be shipped chilled, overnight. Do not ship for Saturday delivery. Culture swabs are unsuitable for cytology.	2 days
Eastern Equine Encephalitis (EEE) Virus qPCR	\$30.00	Fresh or frozen brain (preferable brain stem or medulla) and kidney from a horse; shipped chilled overnight. Brain, liver, heart, or spleen from a bird; shipped chilled overnight. Mosquitos - whole body in pools up to 25. Mosquitos collected within 24 hours and shipped with dry ice (preferred) or ice pack overnight. Mosquitos in RNAlater ☐ shipped with ice pack or room temperature overnight.	1-3 days

Necropsy Exam — Other Animal	\$150.00 per animal plus cremation fee	Fresh carcass of an equine, canine, feline, rodent, or reptile (preferred not frozen) double bagged inside a leakproof cooler; shipped chilled overnight.	20 days
Rabies (No Human Exposure)	\$40.00	Head or carcass (not frozen), double bagged inside a leakproof container; shipped chilled overnight. Requires the examination of a complete cross-section of the brain stem and cerebellum (including vermis, right and left lobes). If the cerebellum is not available, a diagnosis can be made by the examination of the brainstem and hippocampi (right and left).	1-3 days





Pseudorabies in Hunting Dogs

By: Pamela Hunter, DVM, Veterinarian Manager

Pseudorabies has been around a long time and is very prevalent in the feral swine population. Also known as “mad itch,” it is caused by a virus that is shed in the saliva and nasal secretions of infected swine and is spread through oral or nasal contact. The name Pseudorabies came from the symptoms observed in rabbits which are similar to the signs seen in rabies. Adult swine are usually asymptomatic, but the virus can cause abortion, high mortality in piglets, and coughing, sneezing, fever, constipation, depression, seizures, lack of muscle control, circling, and excess salivation in piglets and mature pigs. Mortality in piglets less than one month of age is close to 100 percent, but it is less than 10 percent in pigs between one and six months of age. Though swine (both domestic and feral) are the primary reservoir for this virus, it has been reported in other species such as brown and black bears, raccoons, and coyotes. It is likely that exposure of these animals to the virus is through scavenging infected swine carcasses, inhaling an aerosolized virus, or ingesting contaminated feed or water.

Dogs act as secondary dead-end hosts, contracting the disease directly from exposure to pigs or infected pig products. Incubation periods are on average less than three days. Symptoms include intense itching, jaw and throat paralysis, excessive salivation, howling, and death. In most cases, the animals only live two to three days. There is no evidence that dogs can transmit the virus to other dogs. A veterinarian will make a diagnosis primarily by comparing it to diseases with similar symptoms. With rabies, a dog will attack anything that moves and there is no itching or sudden death. With canine distemper, there is no hypersalivation, sudden death, or personality change and respirator and gastrointestinal signs are common. With poisoning, there is no itching or personality change. By the time lab tests are run to confirm a diagnosis, the animal has died. Although there is a vaccine for pigs, there is not one for dogs. With more than 2,000 feral swine hunters/trappers in the state, precautions need to be taken to prevent infection in hunting dogs.

Tests available at BADDL

BADDL Testing Services			
		Blood in EDTA (purple top) or fresh or frozen urinary bladder, brain, lung, conjunctiva, kidney, or spleen from a canine in a sterile container; shipped chilled overnight.	
Canine Distemper IFA	\$20.00	1 ml of serum from a canine in a sterile container; shipped chilled overnight.	1-3 days
Pseudorabies gB ELISA	\$10.00	1 ml of serum from swine in a serum separator tube (red top/tiger top) or other sterile tube; shipped chilled overnight.	2-3 days
Rabies (No Human Exposure)	\$40.00	Head or carcass (not frozen), double bagged inside a leakproof container; shipped chilled overnight. Requires the examination of a complete cross section of the brain stem and cerebellum (including vermis, right and left lobes). If the cerebellum is not available, a diagnosis can be made by the examination of the brain stem and hippocampi (right and left).	1-3 days

BADDL EVENTS



Vet-LIRN iSeq-Salmonella Project

BADDL's Molecular Diagnostics Section contributed to the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) iSeq-*Salmonella* Project. The Vet-LIRN is managed by U.S Food and Drug Administration's (FDA) Center for Veterinary Medicine. The primary objective of Vet-LIRN is to promote human and animal health by partnering with over 40 state and university veterinary diagnostic laboratories. In 2018, the Vet-LIRN provided samples to eight pilot laboratories for assessment of the utility of the Illumina iSeq platform for bacterial surveillance. The results from the sequencing of these samples will allow Vet-LIRN and the iSeq laboratories the opportunity to assess their capability to test previously characterized isolates and confirm that harmonized methods are working properly within the laboratories. In 2019, four additional iSeq laboratories joined the project and were requested to run *Salmonella* isolates as practice samples.

Samples consisted of *Salmonella* isolates collected during a surveillance study to assess the prevalence of *Salmonella* in canine and feline fecal samples across the United States. New iSeq laboratories received four isolates on agar slants for sequencing using the Nextera DNA Flex library preparation kit in order to verify proficiency before proceeding to the *Escherichia coli* and *Listeria monocytogenes* portion of the project. The run is named as Run 4 to remain consistent with previous laboratory analysis. The team was comprised of Dr. Shipra Mohan, David Simon, Melba Marshall, Alexander Nemethy, and Wando Tirado. The group was recognized at the Food & Drug Administration's (FDA) Honor Awards Ceremony for extraordinary collaborative efforts, contributing to the development of joint strategies to limit the spread of antimicrobial resistance.

SERVICE AWARDS

In recognition of 15 years of service and dedication:

- Christopher Feeney, Lead Laboratory Technician IV, Shipping and Receiving Section

In recognition of 10 years of service and dedication:

- Dr. Shipra Mohan, Microbiology Department Head

In recognition of 5 years of service and dedication:

- Dr. Reddy Bommineni, Laboratory Director/Bureau Chief of BADDL
- Kailyn Feltman Garcia, Biological Scientist IV, Serology Section Head
- Angel Sepulveda, Laboratory Technician I, Necropsy Section

PROMOTIONS

- Susan Soto, Lab Tech IV, Serology Section



REMINDERS

The following dates are upcoming office closures for the Bronson Animal Disease Diagnostic Laboratory:

Monday, January 18, 2021
Dr. Martin Luther King, Jr. Day



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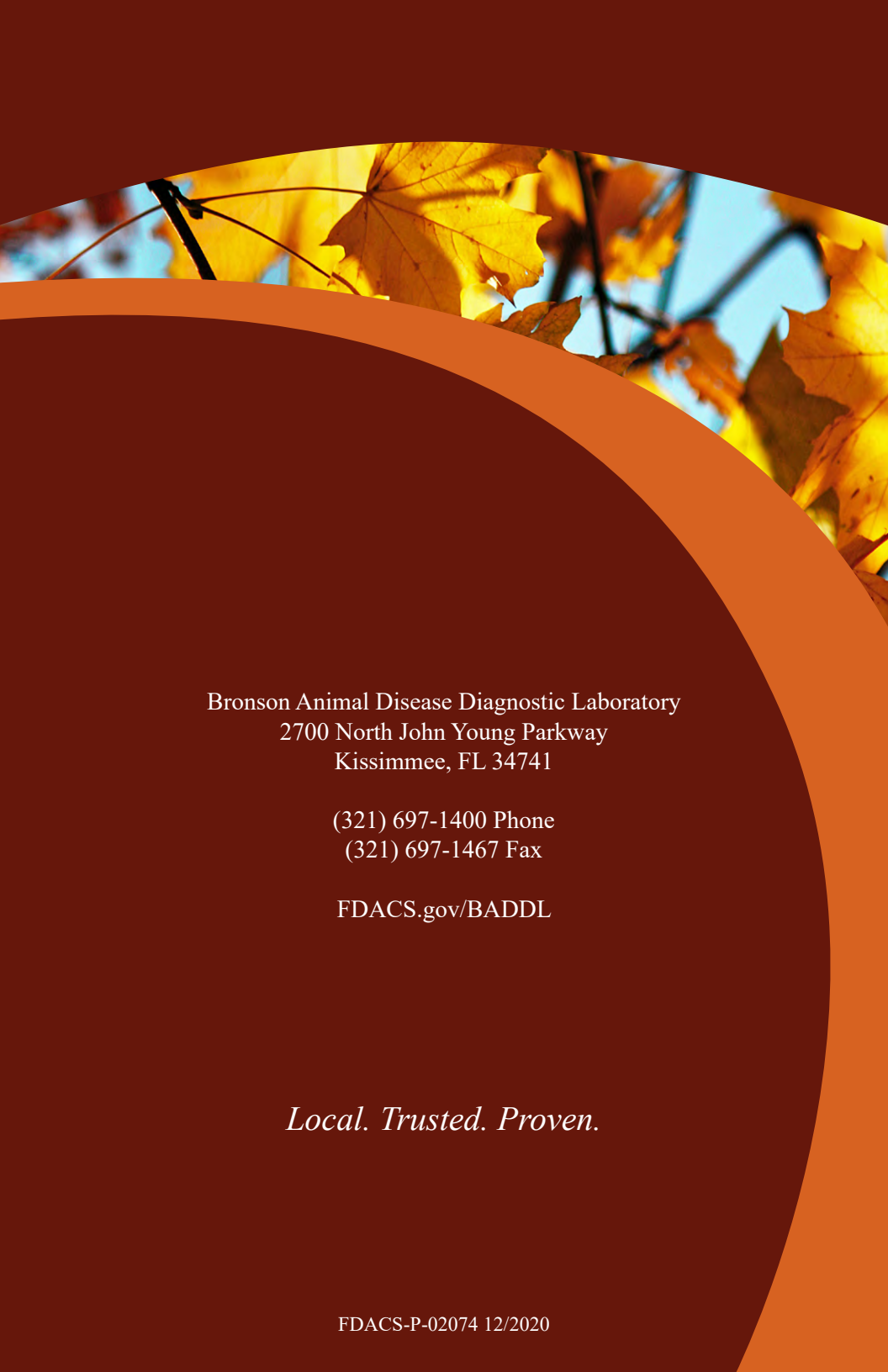
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Any news or information you'd like to see here?
Please contact the newsletter editor, Dr. Karen McKenzie-Alfred, at:
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