OKLAHOMA STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE – OKLAHOMA ANIMAL DISEASE DIAGNOSTIC LABORATORY

OADDL E-News

Winter 2023 • Volume 30

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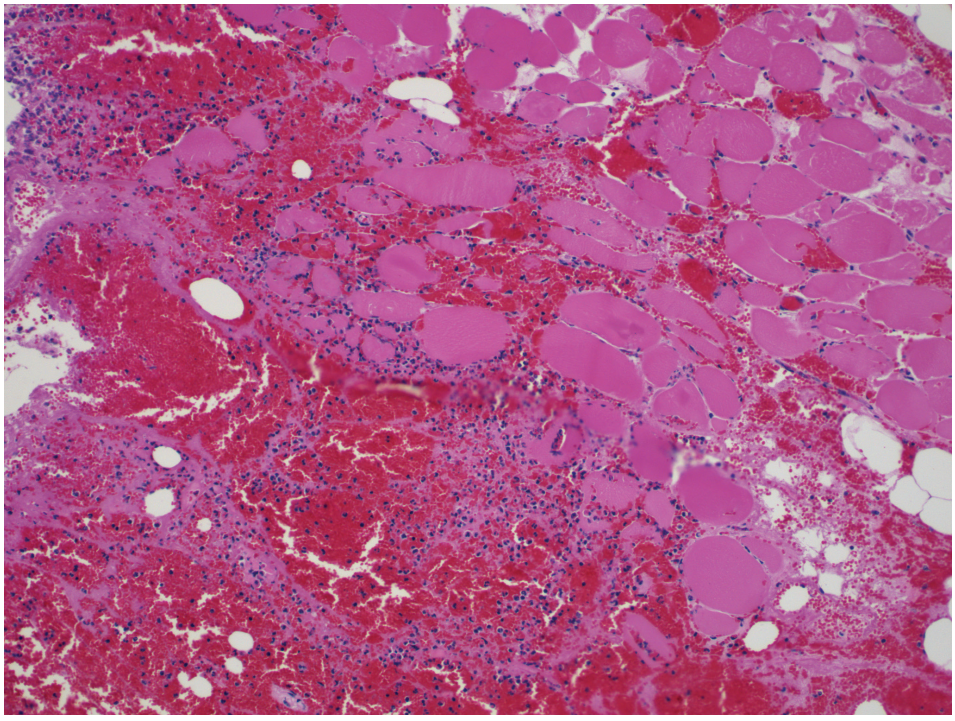

Diaphragm muscle: Myofibers exhibit necrosis. Neutrophilic infiltrates and hemorrhage are additional lesions compatible with Clostridial myonecrosis.

Blackleg: Prototypical Disease - Atypical Presentations - Part 1

Blackleg - or Clostridial myonecrosis - is one of the oldest and prototypical diseases known to our profession. It is caused by the gram positive, spore former known as *Clostridium chauvoei*. Ubiquitous in the environment, this disease agent is often acquired early in life as cattle transition from suckling calves to grazing ruminants. Upon ingestion, spores translocate throughout the body, remaining inactive and silent. Spore activation requires an anaerobic environment, inducing spores to germinate and become vegetative cells producing various toxins. The prototypical lesion that induces an anaerobic environment is a severe bruise to muscle tissue, typically focusing on locomotor skeletal muscle groups of the forelimbs and hindlimbs. Recognition of this acute disease is straightforward, encompassing severe lameness, crepitus in muscle, sudden death, and focal necropsy lesions of black, dry, necrotic muscle with air bubbles.

For part 1 of this disease summary, I will summarize one type of atypical presentation, representing numerous diagnostic encounters within practice and diagnostic lab service across 26 years of experience. One recent diagnostic encounter will be described more fully.

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COLLEGE OF VETERINARY MEDICINE

Blackleg: Prototypical disease - Atypical presentations - Part 1 *(continued)*

Generally, part 1 of this report focuses on atypical skeletal muscle locations for the recognition of a blackleg lesion. Again, the vast majority of acute blackleg cases will be quickly recognized within skeletal muscle groups of the locomotor muscles. However, skeletal muscle exists in many other locales. In my career, I have recognized blackleg lesions in masseter muscle of the head, epaxial muscles, the tongue, and several cases in the diaphragm. The initiating stimulus creating anaerobiasis in these unusual locations is often unknown. Speculatively, and respectively, those stimuli could include traumatic bumps with round bale feeders (masseter and epaxial muscles); biting the tongue; and prolonged exercise on a sunny spring day (possibly inducing diaphragm muscle fatigue, spasm, and an anaerobic environment). The central point in recounting these unusual locations is to encourage the field veterinarian to look more extensively if blackleg is suspected, yet compatible lesions in locomotor muscle groups are not observed.

Recently, I examined a young Angus heifer in excellent body condition with a history of sudden death. The diagnostic examination focused on diagnosing usual suspects: blackleg of the limbs, bloat, or acute pneumonia. After such diagnostic pursuits were negative, deeper examination focused on atypical locations for blackleg. After removing all abdominal viscera, the right upper, caudal diaphragmatic crus exhibited a 6cm long, 1.5cm diameter, cylindrical focus of black, dry, necrotic muscle. This entire focus was excised and split, with one half going to histology and one half going to microbiology.

In the microbiology service, the requested anaerobic culture was surprisingly negative and the fluorescent antibody panel for Clostridia was also negative. However, histology revealed acute myonecrosis, air bubbles, and large rods within the lesion (Figure). Reconciliation of disparate diagnostic test results is as common in diagnostic labs as it is in clinical practice. Priority was given to the histology direct observations. The negative culture results were likely explained by delayed initiation of the culture (some Clostridial agents are quickly overgrown by saprophytes in cases with delayed processing). The negative fluorescent antibody panel has had a recent history of being unreliable; this panel is no longer part of our routine testing.

The primary message of this case series is to look at minor skeletal muscle groups if blackleg is strongly suspected but not observed in routine locations. As a secondary reminder message, diagnostic test results can sometimes be in conflict; experience and judgment provide rational explanatory pathways to arrive at confident diagnoses.

— Timothy A. Snider, DVM, PhD, DACVP
OADDL Pathologist

Overview of Rabies Testing at OADDL in 2022

In partnership with the Oklahoma State Department of Health, OADDL has been providing rabies testing for the state since September 2021. Below is a summary of rabies testing conducted in 2022.

— Shannon Caseltine, Dr. Le Mac' Morris, Dr. Jerry Saliki, Dr. Akhilesh Ramachandran

Image: courtesy of Dr. Hongbo Yu, Dept. of Geography, OSU

ANIMAL	# POSITIVE	# TESTED	# UNSATISFACTORY/ UNSUITABLE	POSITIVE CASE COUNTIES
Bats	**4**	66	3	Pittsburg, Tulsa, Payne
Domestic Cats	**2**	165	3	Logan, Roger Mills
Cattle	**3**	36	0	Caddo, Major, Coal
Dogs	**2**	293	13	Greer
Deer	**1**	2	0	Seminole
Horses	**3**	27	0	McClain, Logan, Cotton
Skunks	**30**	49	4	Haskell, Kingfisher, Sequoyah, Garvin, Ellis, Tillman, Pontotoc, Jefferson, Kay, McCurtain, Beaver, Canadian, Cotton, Oklahoma, Comanche, Tulsa, Washita, Lincoln
Raccoons	**0**	24	2	
Others	**0**	28	2	
Total	**45**	690	27	

Avian and Canine Influenza Cases – 2022

Figure 1: Map showing counties in which Type A influenza was detected.

In 2022, Type A Influenza was diagnosed in birds (Chicken, Duck, Swan, Goose, Guinea fowl), and dogs at OADDL.

The first avian case was detected in March and the virus strains were found to belong to the highly pathogenic H5N1 strain (Eurasian lineage goose/Guangdong H5 clade 2.3.4.4b). A history of exposure to wild birds (waterfowl) was reported in some cases. Clinical signs included sudden death, torticollis, nystagmus, lethargy, tremors, labored breathing, and high mortality. Common gross lesions detected in the birds on necropsy examination included pancreatic necrosis and hemorrhages, pulmonary congestion, petechiations in the subcutaneous areas of the ventral abdomen, thick mucus in the trachea, and edematous intestinal walls with red-tinged mucoid contents. Histologic examination revealed massive pancreatic necrosis and pneumonia as well as moderate congestion and edema.

A positive canine influenza case was diagnosed in early December from Tulsa County. Additional virus typing for strain identification was not performed. Following the initial case, several dogs from the same premise tested positive for the type A influenza virus. Common Influenza virus strains seen in dogs include the H3N2 (avian origin) and H3N8 (equine origin). Clinical history included coughing, tachypnea, pneumonia, ocular, nasal discharge, vomiting and diarrhea with or without fever. Additional lesions found on necropsy examination included: broncho-interstitial pneumonia; hepatic congestion; pulmonary congestion; and right A-V valve degeneration (curious correlation to acute development of a cardiac murmur).

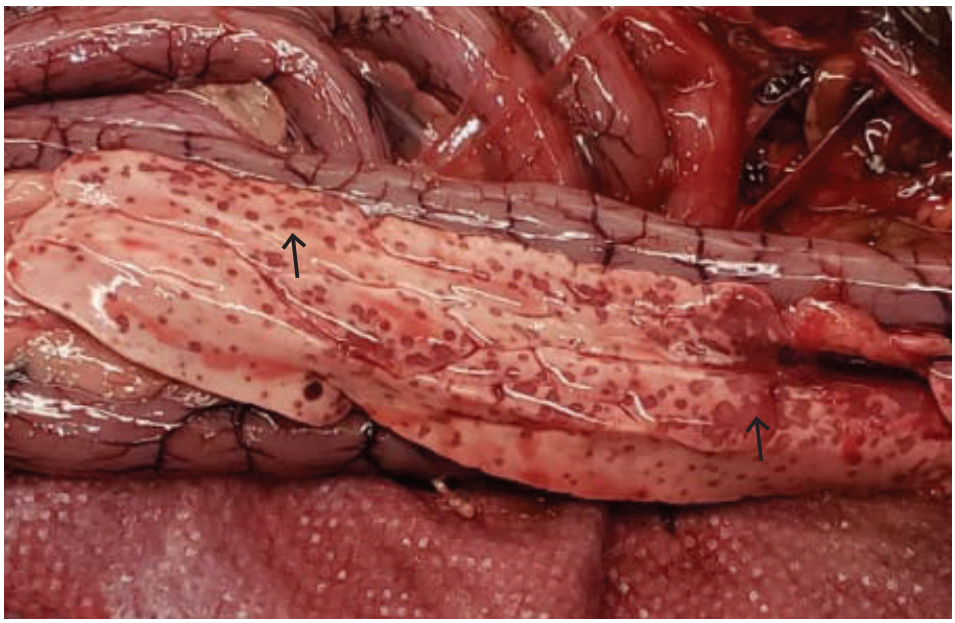

Figure 2: Area of pancreatic necrosis (black arrows) in a Goose.

Canine influenza can spread rapidly in breeding and shelter facilities causing significant morbidity and mortality. Transmission of H3N2 strains from dogs to cats has been reported. The risk of canine influenza spreading to humans is low. No human cases due to canine influenza virus infection have been reported.

— Dr. Sunil More, Dr. Timothy Snider, Robin Madden, Dr. Jerry Saliki, and Dr. Akhilesh Ramachandran

Bartonella-associated Pancarditis in a Dog

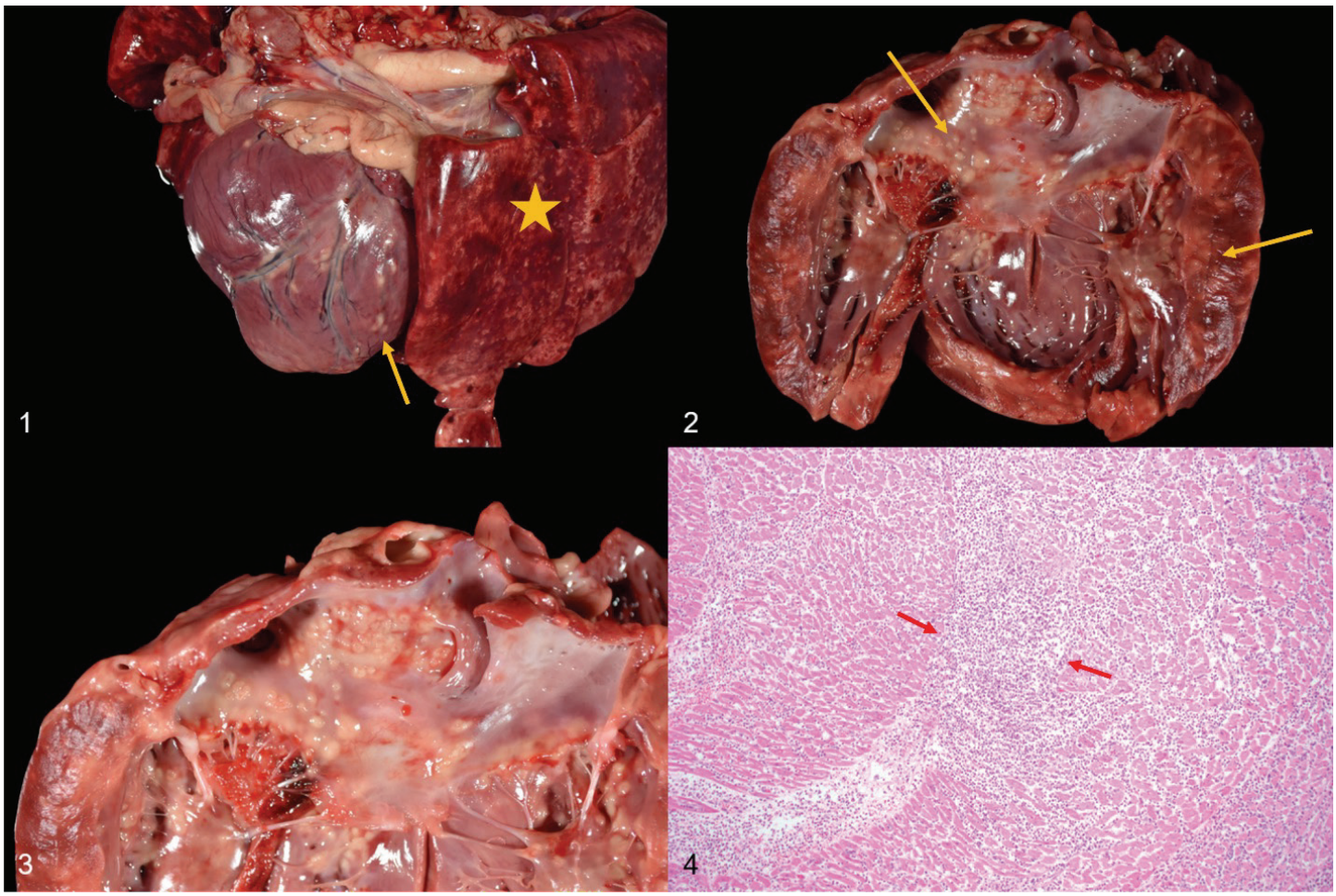

Figure 1. Heart and lung. Scattered on the epicardial surface are pale tan, firm foci (arrow). The lungs (star) are diffusely mottled red to tan. Figure 2. Heart. Upon opening the left ventricle, numerous nodules are scattered throughout the endocardium, valve leaflets, and within the myocardium. Figure 3. Heart. Closer view of the atrial chamber with numerous, variably-sized nodules. Figure 4. Histologic examination of the heart reveals severe pyogranulomatous inflammation (arrows) with degeneration and necrosis of adjacent myofibers.

With Valentine's Day approaching, we present here a case of Bartonella-associated pancarditis identified in a dog at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL). A one-year-old, intact male Border Collie presented to OADDL for necropsy after an acute onset of fever, lethargy, ataxia, and anisocoria. These symptoms began four days prior to necropsy. He improved after two days of antibiotic therapy (enrofloxacin, doxycycline), however the anisocoria returned. Prednisone was added to the antibiotic therapy, and the dog remained bright and alert until two days later when he acutely decompensated and died.

Gross and microscopic examination revealed numerous pale tan foci consistent with pyogranulomas within all parts of the heart (Figures 1-4). There was also considerable myocardial degeneration and necrosis. The lungs were diffusely edematous and effaced by similar pyogranulomatous inflammation. Differentials considered included bartonellosis and other infectious agents such as fungal, protozoal, and parasitic. As no fungal or parasitic organisms were evident microscopically, bartonellosis remained the main differential. Fresh heart submitted to a referral laboratory was PCR positive for *Bartonella* spp.

There are over 20 species and subspecies of *Bartonella*, including *B. vinsonii* and *B. henselae*, the latter being the causative agent of "cat scratch fever". Interestingly, *B. henselae* was named after Diane M. Hensel, a microbiology technologist from Oklahoma who was involved in an outbreak of the disease in 1985. *Bartonella* spp. are gram-negative, fastidious intracellular bacilli that infect erythrocytes, macrophages, and endothelial cells in a variety of species, including humans. Despite its colloquial name, cats are typically not affected by the disease, but rather serve as a source of infection to their human counterparts. In domestic species, transmission can occur via bloodsucking arthropods, particularly fleas. Besides humans and cats, *Bartonella* spp. have been reported to cause disease in dogs and cetaceans, including dolphins, porpoises, and beluga whales. This case is a severe and striking example of an arthropod-transmitted disease and highlights the importance of regular flea and tick preventative in our companion animals.

— Dr. Brianne Taylor, DVM, MS, DACVP

Blood Work Recommendations for Senior Veterinary Patients

As our veterinary patients age, the potential for age-related problems also increases and early clinical signs may not be noticeable to owners. In addition to a thorough physical examination, blood testing is part of a complete health work-up and is necessary for the early detection of disease. **Early intervention and improved quality of life are the goals.** It is important to establish baseline blood work not only to detect problems, but to allow for the evaluation of trends and/or to monitor systemic response to any disease-related treatment. Establishing baseline data can be invaluable when assessing any alterations in a specific blood parameter. The magnitude of change for a particular blood parameter may have more meaning when the change is viewed over time. For example, creatinine concentrations may be creeping upward, but remain within the reference interval. A trend of 2 or more subtle increases in creatinine could result earlier intervention with dietary changes, possible medication changes, and enhanced client education with particular emphasis on recognition of early clinical signs of kidney disease. Indeed, according to the International Renal Interest Society (IRiS), Stage I kidney disease, creatinine and SDMA values are within the reference interval. Other renal abnormalities are present such as abnormal renal palpation or renal imaging, increasing creatinine or SDMA concentrations occurring serially, etc.

For more information regarding IRiS staging http://www.iris-kidney.com/guidelines/staging.html

Points To Consider:

- Diagnostic testing for senior patients should include a complete blood count, a biochemistry profile that includes electrolytes, and a urinalysis. For senior feline patients a thyroid test (total T4) is a standard addition. Abnormalities detected should be followed-up with additional testing as needed.
- Annual or biannual testing is recommended.
- Blood testing should always be performed prior to beginning medications with particular attention to liver and kidney parameters.
- Liver enzyme elevations should always be investigated. These elevations may not be related to primary liver disease, but rather reflect the liver's response to other systemic or metabolic disease. Liver enzymes do not reflect liver function and a more specific test (bile acids) may be indicated. This is especially true for the canine patient on certain medications known to induce liver enzyme elevations in dogs (phenobarbital, glucocorticoids).
- Creatinine and SDMA are indicators of GFR but their relation to kidney disease should be interpreted in light of the patient's hydration status, urine specific gravity, electrolyte parameters, medication history, and/or other medical conditions.

— Dr. Theresa E Rizzi, Dipl. ACVP (Clinical Pathology)

Getting to Know Us

Katelyn Hergenreder joined the OADDL team January 2022. She Graduated winter 2021 with a Bachelors in Nutritional Sciences from Oklahoma State University. In her free time, she likes to sew her own clothes, garden, and go on walks with her corgi Bobo and Husky Silver!

Sara Smith was born and raised in Dallas, Texas. She graduated from Oklahoma State University in the spring of 2021 with a degree in Animal Science: Biotechnology and a minor in Microbiology. Sara joined the Molecular Diagnostic team as a Senior Laboratory Technician in March of 2022. Her hobbies include going on runs, reading a ton of books, playing with her dog, Cash, and horseback riding.

Raina Hahn was raised in Bixby, Oklahoma and joined the OADDL team in May of 2022 as a Senior Lab Technologist for Molecular Diagnostics. She graduated from Oklahoma State University with a degree in Animal Science and a minor in Microbiology. While originally working toward going to vet school, she has found a new interest in research and laboratory testing. She enjoys being outdoors, gardening, hiking, and playing with her dog, Milo.

Maximizing Your Return on Bovine Abortion Investigations

We're in the beginning weeks of the Oklahoma spring calving season. Unfortunately, this also means we will occasionally see reproductive loss due to abortion. This brief article serves as an OADDL specific review for our laboratory users to maximize your return on bovine abortion diagnostics.

Bovine abortion investigations can be challenging and frustrating. This is driven largely by the numerous pathogens and insults leading to abortion and the inability to test all possibilities in a financially responsible way. It is commonly accepted and taught that positive detection of an abortion cause is successful only 25-40% of the time (Snider; OSU Teaching files). While this appears disappointing, it is indeed 'good news' when all testing is negative. The bulk of abortifacient targets we focus our testing upon are agents that often are capable of causing abortion storms.

This article briefly summarizes two main approaches our practitioners can choose from when they have a client asking for diagnostic assistance on a bovine abortion.

The first option is for the aborted fetus to be submitted directly to OADDL for diagnostic necropsy, abortion workup, and disposal. This is our preferred recommendation. When submitting directly to OADDL, our pathologists and staff are able to perform a complete necropsy from start to finish, which yields dividends with respect to comprehensive case consideration, fidelity of samples, and follow through. Our service philosophy with the bovine abortion workup and necropsy is to encourage and to permit the user to choose the discounted package price in lieu of a complex set of a la carte choices that increases the chances something is missed. Our comprehensive bovine abortion workup, available only for whole carcass submittals, is priced at $290 all in. Billed individually, each and every test would amount to over $525, hence the bundled package tests for more causes for less expense. With our bovine abortion workup strategy, we provide a thorough gross examination and report, a comprehensive microscopic investigation and report, lung culture, bacterial cultures for Brucella and Campylobacter, molecular (PCR) testing for IBR and BVD, nitrate levels on ocular fluids, heavy metal testing and trace mineral analysis on fresh liver, and carcass disposal.

Admittedly, large calves are expensive to ship via couriers and many lab users operate cattle ranches in regions geographically distant from Stillwater. Because of those constraints, the second major strategy is for the field veterinarian to perform the gross necropsy, collect the samples, and provide a summation of any gross abnormalities detected. The minimum sample database we would prefer would be as follows: Fixed tissues in 10% formalin (piece of brainstem and cerebral cortex; heart; lung; liver; kidney; spleen; tongue cross section; and abomasum); resected eye for nitrate testing; fresh lung, liver, and kidney (larger samples); and a red top tube full of aspirated abomasal contents. As a reminder, please place the fresh, non-fixed samples in labeled, leak proof containers upon wet ice packs. With those samples, we could replicate all testing from option #1 above except for the gross necropsy report and the disposal. While batch testing discounts are not specifically available for this option, the total fees associated with this approach would be very cost neutral to option #1 because we are not billing for the gross examination and the disposal.

Regardless of the approach, OADDL focuses its testing strategy on high consequence pathogens and insults. Our ancillary testing is focused on specific detection of the following: Campylobacter, Brucella, IBR virus, BVD virus, nitrate toxicity, heavy metal toxicities, and trace mineral deficiencies. Additionally, either approach has open-ended diagnostic approaches where other lesions or pathogens can be discovered, with appropriate follow up options communicated as needed.

To conclude, other important aspects not yet mentioned include performing maternal serology and examination of

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Maximizing Your Return on Bovine Abortion Investigations *(continued)*

fetal membranes. Appropriate testing strategy and selection with these samples can also be important points where abortion causation is discovered. These tests are available through OADDL and are certainly encouraged for pursuit when those samples can be acquired and submitted. Leptospirosis as a cause of bovine abortion can sometimes best be diagnosed via testing of maternal sera. Finally, if the case has unique aspects leading any personnel to suspect less common pathogens or insults, we certainly have access and ability to perform other tests not listed in this article.

— Timothy A. Snider, DVM, PhD, DACVP
OADDL Pathologist

Message from the Director

We are pleased to share with you another quarterly newsletter. 2022 was another great year for OADDL and we are in a strong position heading into 2023, despite headwinds from inflation and hiring/retention difficulties. Noteworthy activities and accomplishments of the previous year include:

- Addition of FedEx as a carrier for our discounted pre-paid shipping method for sending samples to OADDL.
- At the request of the Oklahoma Department of Health, OADDL began testing mosquito pools for surveillance of West Nile virus across the state.
- Enhancing our technical staff in Molecular Diagnostics (see Getting to Know Us)

We look forward with excitement to continue to meet your needs by providing timely and accurate results. This issue of the newsletter includes information on some diseases and guidance on how you can help us to better help you. We hope that this information will be useful to you. Do not hesitate to contact us (oaddl@okstate.edu; 405-744-6623) with any questions or suggestions. Happy reading!

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Ideas/Suggestions for Future Content

We want to hear from you. Send your ideas and suggestions to oaddl@okstate.edu.

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