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VIRGINIA ANIMAL DIAGNOSTIC NEWSLETTER

A joint publication between Virginia Department of Agriculture and Consumer Services and the Virginia Tech Animal Laboratory Services

EDITORIAL

Emerging antimicrobial resistance in Staphylococcus pseudintermedius

First off, I would like to introduce myself, my name is Dr. Jessica Gilbertie and I'm the new clinical microbiologist at ViTALS. Virginia Tech is my alma mater and I'm excited to be back in Blacksburg serving the community for all things microbiology. Apart from service, I will be delivering the microbiology content to the veterinary students at the VMCVM and conducting academic research. My research focuses on biofilm pathogenesis and novel antimicrobial agents.

I started this new role in June of this year. Since starting one of my major observations has been the rising antimicrobial resistance in *Staphylococcus pseudintermedius*. Not just the rising rates of methicillin-resistant isolates (also called MRSP) but multidrug resistance (MDR) and even some that are resistant to every available veterinary antibiotic. For example, 10 years ago in 2014 we observed a 12.7% rate of MRSP compared to a 45.6% rate of MRSP in 2022. In addition, only 17% of *S. pseudintermedius* isolates were considered multi-drug resistant in 2014, while 75.7% are showing MDR in 2022. In the last 6 months, we have identified over 10 isolates that I would consider pan-resistant meaning they are resistant to every antibiotic currently available on the canine antibiotic panel. This means that clinicians are forced to reach for human antibiotics such as vancomycin or linezolid. Unfortunately, we do not have canine specific breakpoints for these antibiotics leading to extrapolation from human breakpoints. This raises concerns for antimicrobial stewardship and One Health.

With significantly high rates of AMR in the most commonly isolated bacteria from small animal medicine, we plan to start researching these isolates in depth and comparing our results to other clinical laboratories across the United States. This is also prompting the need to investigate new antibiotics

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that could be used, either new or repurposed, which is now a major project in my research laboratory. We are eager to build our database of these highly resistant clinical isolates for study. If you are interested in helping us understand the burden of MRSP and MDR *S. pseudintermedius* in Virginia or across the country please contact me at jmg1329@vt.edu. We are would love for you to send us isolates or chat about the burden of MRSP and MDR affecting your patients.

Jessica Gilbertie MS, DVM, PhD - Virginia Tech

Horses and Donkeys

Equine motor neuron disease

A two-year-old Quarter horse filly was euthanized following a course of progressive neurologic clinical signs which included ataxia with neurolocalization to C5/6-T2/3. Postmortem examination revealed mild articular cartilage degeneration within the cervical articular facets, but no evidence of spinal cord compression. A spectrum of lesions were detected microscopically in the neuraxis, including chromatolysis and degeneration of neurons within the spinal cord ventral horn and brainstem motor nuclei associated with axonal swelling in the affected gray matter. These findings were compatible with a diagnosis of equine motor neuron disease, a term that encompasses a group of neurodegenerative diseases through to result from primary metabolic dysfunction of the nerve cell body. In some cases, vitamin E deficiency appears to contribute to the pathogenesis.

Thomas Cecere DVM, PhD, DACVP - Virginia Tech

Osseous cyst-like lesions in a mare

A 24-year-old Arabian mare was euthanized because of a history of chronic right forelimb lameness, attributed to carpal effusion. She was used primarily as a broodmare. The gross examination was unremarkable other than mild swelling in the periarticular tissue surrounding the right carpal joint and slight roughening of the articular surfaces of the middle carpal joint. Longitudinal sectioning of the third carpal bone and third metacarpal bone revealed four, 2-3 mm diameter, red cavitated areas in the third carpal bone, near the distal articular surface. Histologic examination of a section of decalcified third carpal bone revealed that the bony trabeculae were multifocally effaced by nodular aggregates of fibroblasts and collagen surrounding pools of fibrillar amphophilic material, with scattered neutrophils and lymphocytes. These lesions are most consistent with osseous cyst-like lesions, previously called subchondral bone cysts. These cystic lesions can be developmental or secondary to trauma. Osseous cyst-like lesions are more common in male horses, and the most frequently affected site is the medial condyle of the femur. Clinical signs range from mild to severe lameness. Since the cavitated areas are not lined by epithelial cells, these lesions are not true cysts.

Teresa Southard DVM, PhD, DACVP - Virginia Tech

Bovine and Camelids

Hypovitaminosis A and E in a calf

A field necropsy consisting of lung, liver, and kidney from a 2-week-old Angus heifer calf was presented for diagnostic investigation. The fall calving herd had multiple weak calves born that died anywhere from a few hours to several weeks

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after birth despite interventions. Routine testing, consisting of sub-gross and histopathologic examination, as well as several cultures and PCR tests, revealed no significant abnormalities. A liver sample was sent for vitamin and mineral analysis. The sample was found to be deficient in vitamin A at 8.668 ppm (normal range 28-70 ppm) and vitamin E at 1.886 ppm (normal range 2.5-3 ppm). Vitamin A and E have a wide range of functions in the body, and deficiencies in either or both are associated with similar herd problems. Fresh, green grass is the best source of both for the cow, who then passes it to the calf via milk consumption; however, much of Virginia has battled drought conditions this year, potentially compromising forage quality and vitamin intake.

Taylor Young, DVM - VDACS Lynchburg RAHL

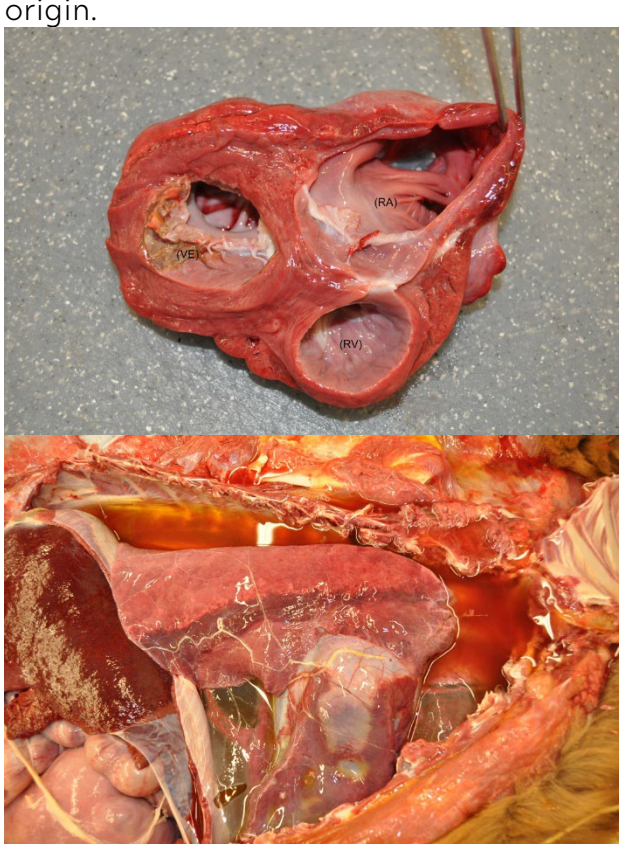
Salmonella bovine abortion

A 6-month gestation Holstein heifer fetus was submitted for abortion investigation along with placenta and maternal serum. The herd had experienced sporadic abortions at a similar time in gestation over the last year. Gross examination of the fetus revealed extensive purple, gelatinous edema in the subcutaneous tissues of the trunk, and the pleural, peritoneal, and pericardial cavities contained excessive amounts of serosanguinous fluid. Histopathology identified mild mineralization and trophoblast necrosis in the placenta. An abortion PCR panel was run on liver, abomasal fluid, and placenta, and *Salmonella* was detected from the placenta. Follow-up culture grew a group C isolate from both the liver and the placenta. *Salmonella* is a known cause of infectious abortion worldwide.

Taylor Young, DVM - VDACS Lynchburg RAHL

Chronic cardiomyopathy and valvular endocarditis manifesting with respiratory signs in an Alpaca

A 3-year-old, pregnant Alpaca was presented with a history difficult breathing for approximately 6 weeks. The alpaca received a transfusion and a presumptive diagnosis of gastrointestinal ulceration was made. The animal was seemingly better after treatment but died shortly after and was submitted for a necropsy. On gross examination, the abdominal and thoracic cavities contained very large amounts of fluids and the lungs had bilateral ventral atelectasis (left image) affecting approximately 40% of the pulmonary mass. The liver had a very enhanced reticular pattern which, along severe ascites and hydrothorax suggested possible heart failure. Examination of the heart (right image) revealed a very dilated right auricle (RA) and right ventricle (RV) with thinned right ventricular wall and a severe, chronic, mitral valve vegetative endocarditis (VE), likely of bacterial origin.



Santiago Diab DVM, DACVP - Virginia Tech

Disseminated histophylosis in a heifer

A 7-month-old, heifer was submitted for a few day history of being down that progressed to an inability to swallow. The heifer was treated for possible Listeriosis but had no improvement. At necropsy, the heifer had acute meningoencephalitis, multiple chronic cardiac abscesses with vasculitis and thrombosis, and multiple renal infarcts. The heifer was negative for rabies. Although there were no significant findings on cultures, a respiratory PCR panel detected *Histophilus somni* in the heart and kidney. *Histophilus somni* is a common bacterial

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pathogen affecting cattle and is commonly associated with septicemia. Thrombosis is also a common feature of this infection. Clinical signs can vary depending on the organ(s) affected but may include sudden death, lethargy, ataxia, lameness, fever, and respiratory symptoms.

Figure 1 Spinal cord. Meningitis

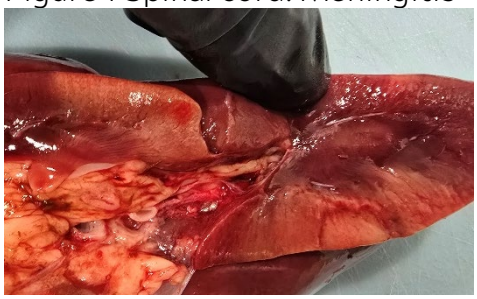

Figure 2 Kidney. Multiple infarcts

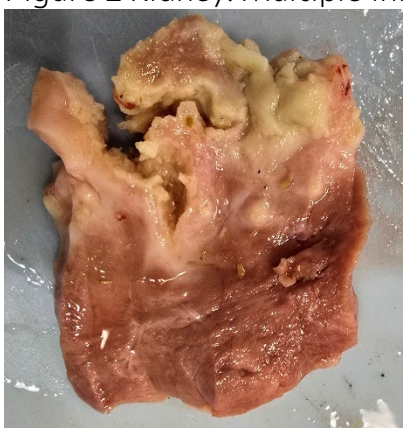

Figure 3 Heart abscess with fibrosis

Jaime Weisman DVM, MS - RAHL Warrenton

Presumptive P. tenuis cerebral migration in an alpaca

A 6-year-old, male alpaca presented to the teaching hospital with a history of ataxia and weight loss, as well as blindness in the right eye and a melting ulcer in the left eye. The alpaca had been previously treated for meningeal worm. Physical examination findings included fair body condition, quiet but alert mentation, absent menace response in the left eye and intact palpebral reflexes bilaterally. The animal was euthanized due to poor prognosis. Gross examination confirmed the melting ulcer in the left eye, but there were no other significant findings. After fixation, the brain was sectioned and the lateral ventricles, third ventricle, and mesencephalic aqueduct were moderately dilated and the pineal gland was enlarged and gritty. Histologic examination revealed a linear track of hemosiderin-laden macrophages, large numbers of eosinophils, and plasma cells in the midbrain and clusters of foamy macrophages multifocally surrounding parenchymal vessels. The pineal gland contained aggregates of mineral. There were also clusters of pigmented macrophages in the pituitary gland. The linear lesion in the brain with associated eosinophils is consistent with parasite migration, most likely *Parelaphostrongylus tenuis*. Inflammation in the midbrain and pituitary gland, along with the mineralization of the pineal gland, likely caused obstruction of cerebrospinal fluid flow, resulting in hydrocephalus, as well as cranial nerve damage.

Teresa Southard DVM, PhD, DACVP - Virginia Tech

Avian

Avian gastric yeast in two budgerigars

Two 3-year-old male budgerigars from a large public aviary were submitted for post-mortem examination due to a history of lethargy, regurgitation, and weight loss despite treatment interventions. In both birds, gross examination revealed abundant mucus in the proventriculus that corresponded histologically to proventriculitis and ventriculitis associated with avian gastric yeast, particularly in the transition zone from the proventriculus to the ventriculus (isthmus). Avian gastric yeast has a characteristic histomorphology of elongate, rod-shaped yeast organisms that are often

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arranged perpendicularly to the mucosa and linearly stacked. This organism, known as *Macrorhabdus ornithogaster*, can cause excess mucus production and enlargement of the proventriculus, proventricular and ventricular erosions to ulcerations, and disruption of the ventricular koilin layer. These pathologic changes cause a range of clinical presentations from mild subclinical disease, to acute anorexia and regurgitation with death in 24-48 hours, or chronic regurgitation, diarrhea, and weight loss. Budgerigars are frequently severely affected, and infection of the whole flock can occur with chronic presentations and intermittent acute episodes. This disease hinders effective digestion, causing affected birds to lose weight despite increased feeding regimens. This organism is highly contagious and transmitted through direct contact with infected droppings or feed material.

Alexandra Reddy, DVM - Virginia Tech

"Lock jaw" syndrome in a cockatiel

A 3-month-old female cockatiel developed what the owner described as 'lock jaw' and died later that day. This was the second bird in the group to die with these signs. Significant macroscopic findings at necropsy included the presence of tenacious viscous fluid within the nares and yellow-tinged exudate adherent to the medial canthus of both eyes. Histologic examination revealed heterophilic and necrotizing infraorbital sinusitis and myositis with mixed bacteria present. These findings were compatible with lockjaw syndrome described in juvenile cockatiels. (Avian Pathology (2001) 30, 49-53). A number of different bacterial pathogens have been isolated from birds with this syndrome, including: *Pseudomonas, Staphylococcus, Streptococcus, Bacillus, Klebsiella, Mycoplasma spp., E. coli*, and *Bordetella avium*.

Thomas Cecere DVM, PhD, DACVP - Virginia Tech

Companion Animals

Cutaneous oomycete in a Great Pyrenees dog

A 2-year-old male neutered Great Pyrenees dog presented to the dermatology service for a non-healing ulcerated lesion on high right hind leg in August of this year. Several biopsies of the area were taken as well as a fine-needle aspirate. On cytology, fungal hyphae were noted. Further supporting the cytology results, histology revealed intralesional hyphae that were suspected to be an oomycete based on their appearance on a GMS stain. A fungal culture was submitted that revealed an unusual colony morphology that could not be identified by lactophenol preparation. Therefore, it was elected to work with the University of Florida Molecular Fungal ID Lab under the guidance of Dr. Aline Hoffman. Sequencing of the isolate revealed the oomycete *Paralagenidium karlingii*. The patient was started on a course of minocycline as the owner elected for medical versus surgical management. Minocycline was chosen based on case reports of tetracycline activity against these isolates. As of November 11, 2024 the patient is responding well to therapy with a reported 50% reduction in lesion size and absence of any ulceration. On repeat cytology, via GMS stain hyphae were still observed by appeared in significantly lower numbers. The treatment is planned to continue for several more months.

To note, oomycosis is not a fungal infection commonly found in Virginia. This disease, caused by *Pythium, Lagenidium* or *Paralagenidium* spp. is more often associated with the Gulf Coast. It is important to note the possibility of these organisms now being present in cutaneous infections of the Mid-Atlantic region.

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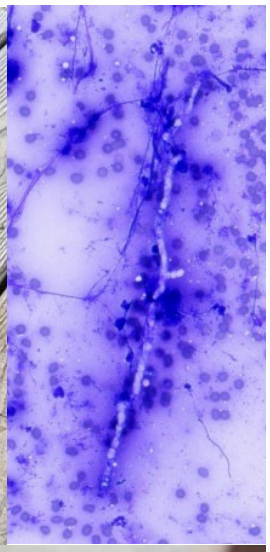
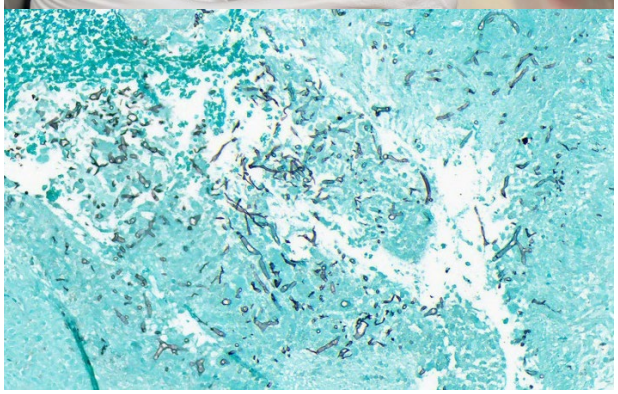




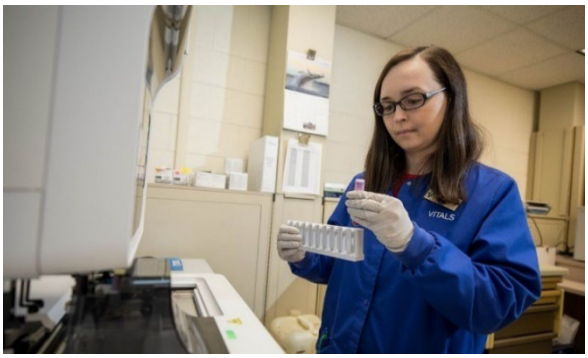
Jessica Gilbertie MS DVM PhD - Virginia Tech

Gastric Dilation and Volvulus in a Dog

A 9.5-year-old neutered Sarplaninac was submitted for necropsy with a history of being found deceased with no current illness. He had a period of vomiting 3 weeks prior for 3-4 hours and since then has had no other health concerns. On gross necropsy the abdomen was very tight. Upon opening the abdomen, the stomach had a 270-degree torsion and severe splenomegaly and distention of the vascular system. Diagnosis was a gastric dilation and volvulus or GDV. This is a severe condition where the stomach fills with fluid or gas and expands and then rotates. This is most common in large, deep chested dog breeds and while the exact cause is unknown risk factors include exercise after eating, drinking excessively, stress or anxiety. Symptoms include a bloated abdomen, and nonproductive retching. Treatment requires immediate surgery with a generally good prognosis when found quickly.

Jamie Horstmann Blinn DVM, RAHL Harrisonburg

LABORATORY NEWS



ViTALS

Kevin Lahmers, Francisco Carvallo, Santiago Diab, Anna Hassebroek, Abraham Adeyemo, Jessica Gilberte, Lauren Lytle, Alex Fox, and myself traveled to Nashville in October for the Annual USAHA/AAVLD meeting. The meeting gave us the opportunity to visit with colleagues from laboratories, and state and federal programs from around the country, and inspired us to bring knowledge and new ideas back to ViTALS. I was privileged to have to opportunity to be Program Chair this year, and got a backstage view of the amazing work that our colleagues do. Our faculty served as abstract reviewers (Lahmers, Diab), session moderators (Diab, Gilberte), session organizers (Carvallo), and invited speakers (Lahmers). Catie Burgess from the Lahmers lab won the Epidemiology Travel Award to present her work on Theileria orientalis. The meeting always has the effect of allowing us to learn new things and get excited about the great work that we all do. As we head into the winter season, I am thankful for those

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those that dedicate their lives to animal health and serving animal owners in the Commonwealth of Virginia, and surrounding States. I hope everyone takes some time for themselves to recharge over the holiday season.

Tanya LeRoith DVM, PhD, DACVP. ViTALS Director

VDACS

Testing for Highly Pathogenic Avian Influenza Virus in milk has continued at the Harrisonburg Regional Animal Health Laboratory, with over 240 samples tested to date (all testing negative). Federal orders have restricted interstate movement, enhancing surveillance through pre-movement testing of lactating dairy cows. Testing has also continued for Avian Metapneumovirus, with over 6,600 samples tested to date. In Warrenton, analysts are successfully working on bringing in CL SHI testing capabilities and are continuing to maintain a heavy necropsy and CEM culture load. The Wytheville Laboratory is continuing the last testing protocols in its annual fish testing in support of Virginia's Department of Wildlife Resources and some limited private producers. This testing covers both diagnostic and regulatory testing for fish health in the Commonwealth and surrounding states. Necropsy workload has remained steady, and serology testing has remained heavy. The Lynchburg Laboratory has seen a steady volume of necropsies after resuming this service in November 2023 and has been maintaining a steady workload regarding to PCR and Food Safety testing. In FY2024, over 5,000 samples were tested in the Food Safety Laboratory in support of private and commercialized producers.

Initial schematic design plans for the expansion of Warrenton, Harrisonburg, and Lynchburg Regional Animal Health Laboratories were submitted to the state's Department of Engineering and Budget for review and approval. A total of 8,800 sq feet will be added between the three laboratories.

VDACS OLS hosted the biennial audit and maintained accreditation of all four Regional Animal Health Laboratories (RAHL) from the American Association for Laboratory Accreditation (A2LA) through 2026, and brought all National Animal Health Laboratory Network (NAHLN) testing onto the A2LA scope for accreditation. Individual Standard Operating Procedures (SOPs) for the NAHLN testing performed at multiple sites were combined into one system SOP instead of having duplicate SOPs based on location. Testing additions and changes bring the total accredited testing methods up to 17 for Harrisonburg, 19 individual tests and one panel consisting of 8 pathogens for Lynchburg, 7 for Warrenton, and 15 for Wytheville, comprising 71 SOPs. These testing methods and additional SOPs must maintain all the requirements of the ISO 17025:2017 standard requiring continued monitoring via our Quality Management System and are subject to rigorous internal audits to ensure the quality standards are met.

New hires in the system include a new administrative and laboratory assistant in Warrenton- Barbara Rocha, and a new serologist in Harrisonburg- Allison Sanford. An internal transition occurred with Gillian Slekar moving into the Harrisonburg Dairy Microbiologist and Laboratory Evaluation Officer position.

We are always open to feedback and ideas for testing to further assist our agricultural community. It is our goal to make this lab system the best it can be to further serve our clients. Our partnership with the Virginia Tech Animal Laboratory Services Lab (ViTALS) continues to evaluate ways to collaborate and expand testing services. Whether by utilizing data for reports or enhancing testing capabilities, we look forward to continuing to work together and with all of you.

Jessica Walters DVM, PhD, DACPV. Program Manager, Office of Laboratory Services

LABORATORY LOCATIONS

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