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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

Review of BVD presentations seen recently in Southwest Virginia

Bovine Viral Diarrhea (BVD) is a disease that was first identified in the 1940s as a gastroenteritis with severe diarrhea, ulcerations of the muzzle, nasal and oral cavities, fever, leukopenia, immune suppression, and abortions. Despite effective vaccines and knowledge of the disease, BVD is still responsible for major economic losses in the beef industry today. The virus is mainly transmitted in feces and secretions from the nose and mouth. It often enters a herd through untested herd additions, fomites (contaminated OB sleeves, boots, equipment), and fence line contact with neighboring cattle. There are two types of infection: Transient (TI) or acute and Persistent (PI) or chronic infection. Transient infections are acquired after birth, last a few weeks and cattle mount an immune response to clear the virus. TI infections account for 95% of BVD infections but are a minor source of spread of virus in the herd. PI infections are acquired in utero-fetal exposure to BVD during 60-120 days of gestation. PI animals never mount an immune response and are infected for life. PI animals account for less than 5% of BVD infections but are a major source of spread within herds. Timing of gestational exposure results in different outcomes. At fertilization, conception rates may be reduced and animal rebreeds. At 0-45 days, usually result in fetal death. At 60-120 days, if fetus survives it will be persistently infected. After 120 days, calf may be aborted, stillborn, have congenital defects (cerebellar hypoplasia, retinal atrophy, microphthalmia, cataracts), or be born normal. BVD testing here at the lab is antigen based, either "ear notch" or serum, which are both ELISA tests. Differentiating between PI and TI can be difficult since PI calves do not mount an immune response. Typically, if we think of BVD in feeder cattle, primary presentation is pneumonia. Below are 4 cases we saw here at the lab. The first two cases involve co-mingled feeder calves for backgrounding, the other two were non typical presentations.

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Case 1: 7/24/23

Group of 70 stockers purchased at various livestock markets beginning the first of July. This was the 8th calf lost despite veterinary directed treatment, little to no response to treatment. Gross necropsy: 500 lb heifer, primary pneumonia supported by histopathology. Respiratory PCR Panel detected *Mycoplasma, Pasteurella multocida, Mannheimia haemolytica* and bovine respiratory syncytial virus. *Theileria* ssp. was also detected. They continued to lose calves despite treatment, decided to ear notch the remaining 57 to try and identify a PI, 31/57 came up positive. Recommended to re-notch positives in 3-4 weeks distinguish between PI and TI. Unfortunately, no follow up.

Case 2: 10/4/23

Group of 166 stockers, 10th animal to die presented for necropsy, well managed operation, routinely "ear notch home test" high risk and non-source verified cattle. This was the last group assembled and did not test on arrival. This 650 lb beef heifer had been purchased (livestock market) 2 weeks previously and worked coming off the trailer. Animals are exhibiting respiratory signs, fever, lethargy and clear profuse diarrhea with little to no response treatment. Producer suspected a PI in the group. Primary pneumonia grossly, supported by histopathology, *Mycoplasma bovis* and *Mannheimia hemaolytica* detected on PCR respiratory panel. This calf tested negative on ear notch, positive on serum, retested due to history and gross findings. The producer tested the rest of group and found 2 PI calves; continued losses for 2 weeks post removal. Death loss just under 25%.

Case 3: 6/26/23

Yearling beef heifer presented after brief illness, failed to respond to treatment. She was raised as an orphan calf from a neighbor. This heifer was in excellent body condition with a shiny sleek haircoat. Grossly the trachea/lungs contained a fungal plaque just distal to the epiglottis, extending down into the bronchioles. Cranio-ventral lung lobes had a moderate line of demarcation. Inflammation of the small intestine and spiral colon. Histopathology findings were multifocal fibrinous and lymphohistiocytic interstitial pneumonia with severe diffuse ulcerative enterocolitis. Ancillary testing: positive ear notch, negative on culture and respiratory panel. *Aspergillus* spp. was confirmed on fungal culture.

Case 4: 6/23/23

An 11-month-old steer found dead suddenly. This calf had been born on the farm and was being backgrounded. This herd consisted of 40 "Momma" cows and the producer had lost 11 calves during the past year. Grossly this calf had a rough haircoat, small for its age, manure caking of the perineum, front claws elongated, proximal hoof wall ridges, coronary band ulcer, oral ulcers, abomasal ulceration and spiral colon/colon inflammation. Lungs appeared grossly normal with no growth on culture. Histopathology reported multifocal acute necrosis in the abomasum, acute ulcerative colitis and no abnormalities in the lungs. Ancillary testing-positive ear notch. The chronicity of this case was interesting to observe and makes one wonder about the degree of infection on the farm. Unfortunately, no follow up.

Tom Lavelle DVM, Wytheville Regional Animal Health Laboratory - VDACS



Left: multifocal oral ulcers. Right: coronary band ulcer.

Horses and South American Camelids



Cerebral cryptococcus in a horse

A 17-year-old Hanoverian gelding was found down and demonstrating seizure activity. Seizures continued despite treatment and the owners elected humane euthanasia. On postmortem examination the choroid plexus appeared diffusely thickened and red to brown. In the heart, there was multifocal to coalescing subendocardial mineralization of the left ventricle; no other abnormalities were found. On microscopic examination, the meninges in all examined sections were variably thickened by a dense inflammatory population centered on few fungal yeasts. As seen in this 20x image of cerebellum, the inflammation is composed of many macrophages, lymphocytes and few multinucleated giant cells. Fungal yeasts (arrow) were approximately twice the size of a large lymphocyte and frequently surrounded by a peripheral clearing up to 10 µm wide. Yeasts stained positive with Periodic Acid-Schiff (PAS) histochemical stain and the clear capsule stained positive with Mucicarmine stain. A diagnosis of Cryptococcal meningitis was made. Cryptococcus sp. yeast are found most commonly in avian feces (e.g. pigeons) and are transmitted by inhalation. In horses, this fungus has been reported to cause rhinitis, pneumonia, encephalitis and meningitis. Spread to the central nervous system (CNS) may occur hematogenously or via lymphatics from primary sites of infection, or via local extension through the nasal cavity. There was no evidence of fungal infection or granulomatous inflammation in any other organ system in this patient.

Anna M Hassebroek, DVM, MPH, PhD, DACVP, Virginia Tech



Granulomatous inflammation in the meninges. Arrow is pointing to a fungal yeast structure.

"Alpaca Fever"

An 8-year-old female alpaca presented after being euthanized for a chronic presumptive uterine infection and inability to rise. The alpaca was in poor body condition. The thoracic cavity contained a large amount of yellow, clear fluid, had mild pleural adhesions and a small lung lobe torsion along the right cranioventral margin. The peritoneal cavity revealed a severe peritonitis, with severe fibrinous adhesions and two large abscesses present in the retroperitoneal space. *Streptococcus equi* ssp. *zooepidemicus* and *Escherichia coli* were cultured from the abscesses. *Strep. zooepidemicus* is the causative agent of a disease known as "alpaca fever," and is associated with pleuritis and peritonitis, as seen in the case.

Taylor Young, DVM - Lynchburg Regional Animal Health Laboratory, VDACS



Abundant fibrin and pus in the serosa of the intestinal loops (yellow material).

Ruminants



Arsenic intoxication in cows

Two 8-year-old female angus cattle were submitted for necropsy after becoming acutely down and dying or being euthanized due to poor quality after 24-48 hours. This farmer had lost 6/10 cows of his adult herd, but his calves did not seem affected. Some cows were treated for grass tetany with only one cow recovering. During the necropsy, both cows were in good body condition with moderate to severely enlarged livers and severally enlarged gallbladders with extremely thick bile. The abomasum contained mudlike contents and 1-2 small pieces of what appeared to be charcoal. At this point, toxicity was high on the differential list and the producer was asked if there was access to a burn pit. The cows did have access to a burn pit and were noted to aggressively attack the mineral when put out. Referral testing was sent out and arsenic toxicity was confirmed. Arsenic can be found in many sources, including ashes from burned pressure treated lumber, lead arsenate, old pesticides, older drugs, drilling muds and soils that are contaminated. The burn pit is the most likely source of arsenic for these cases. Blood arsenic for other animals in the group can be used to determine who was exposed. Arsenic excretion is variable due to the ingestion, residence time, age of the animal and disease status and withhold times are estimated to be 6+ months.

Jamie Horstmann, DVM - Harrisonburg Regional Animal Health Laboratory - VDACS

Blackleg lesion in the mammary gland of a calf

A 5-month-old female calf succumbed to Blackleg disease with all the classic lesions of blackleg in different skeletal muscle groups and the heart. Interestingly, a single circular, approximately 7 cm diameter, well demarcated lesion was observed in the parenchyma of the left, caudal mammary gland. This lesion was dark red and edematous. Histology of the mammary gland showed a focal, fibrinoneutrophilic mastitis with intralesional Gram + bacilli, compatible with *Clostridium* sp. Immunohistochemistry for *Clostridium chauvoei* was positive in the mammary gland, skeletal muscle and heart, confirming the common etiology for the lesions in these tissues. Lesions of blackleg in tissues other than skeletal muscle, heart and pericardial sac are very uncommon but, as demonstrated in this case, may occur.

Santiago Diab DVM, DACVP. Virginia Tech



Skin and mammary tissue with a focal area of inflammation.

Aortic aneurysm in a Holstein cow

A 6-year-old Holstein bovine presented for necropsy following a 1-day duration of not doing right. At necropsy there was abundant blood within the abdominal cavity. The specific location of the hemorrhage was not identified but rupture of the abdominal aorta was suspected. Microscopically, there was no evidence of an infectious, inflammatory, degenerative, or neoplastic process and all tissues were within normal limits. Based on

the gross necropsy findings and lack of other findings, an abdominal artery aneurysm/rupture was favored. According to one report, abdominal artery aneurysms appear to primarily affect 2.5-5.5-year-old Holstein cattle. It has been predominantly reported in female Holstein cattle but this trend may be associated with the fact that female dairy cattle outnumber males. The cause for abdominal artery aneurysms is still unclear but chronic damage to affected vessels has been noted in one study.

Jaime Weisman, DVM, MS - Warrenton Regional Animal Health Laboratory - VDACS

Avian



"Swollen Head Syndrome"

Broiler breeder hens were submitted with complaints of neurological issues and elevated mortality. One exhibited a tremor, others exhibited paroxysmal torticollis. They had swollen heads with non-pitting edema, and some exhibited harsh respiratory noises. On necropsy, significant gross lesions included actively regressing ovaries, edema in soft tissues of the head, and soft, fragile craniums. Blood smears indicated an inflammatory response with monocytosis and elevated heterophil count with a left shift. Avian influenza, Newcastle, and mycoplasma PCR tests were negative. Pure E. coli was isolated from the brain of one of the birds. Trachea and nasal turbinate tested positive for Avian Metapneumovirus subtype B at a referral laboratory. Avian metapneumovirus affects numerous species of birds, with turkeys most severely affected. In chickens it and E. coli are associated with "swollen head syndrome". While subtype C was

identified in the USA in the 1990s, before 2023 subtype B had not been of concern in this country.

Hailey Quercia DVM, MS, DACPV - Harrisonburg Regional Animal Health Laboratory- VDACS

Insecticide intoxication in chicks

One week old chicks were submitted, with the complaint of chicks holding their heads back with eyes shut, and high mortality. On exam the eyelids could be gently opened, showing swollen conjunctiva and excessive lacrimation. Menace response was largely absent, pupillary light reflex and palpebral reflex was present. Chicks wandered away from flock mates and did not seek out shelter. On necropsy the most significant gross lesions were enlarged livers and large amounts of darkling beetles in the GI tract. Throughout the necropsy a strong metallic-like burning odor was present. Testing of GI content at a referral laboratory revealed high levels of insecticide. The flock was monitored, and both mortality and insecticide levels dropped as the birds aged. A possible exposure route to the chicks would have been through eating beetles which had been targeted with the pesticide.

Hailey Quercia DVM, MS, DACPV - Harrisonburg Regional Animal Health Laboratory- VDACS

Companion and Exotic Animals



Protothecosis in a dog

A 4-year-old, castrated male, Rhodesian Ridgeback dog presented to the Virginia Tech Veterinary Teaching Hospital for further evaluation of a 9-month history of large bowel diarrhea, minimally responsive to antibiotics, diets, and anti-inflammatory doses of prednisone. His physical examination revealed generalized

muscle wasting (BCS 4/9 and MCS 1.5/3), pale mucous membranes, severe hematochezia with mucus and mucosal sloughing on rectal exam, multifocal lesions in the tapetal fundus of both eyes, and a large subretinal granuloma on the left eye. His complete blood count and chemistry panel revealed, most notably, moderate renal azotemia (BUN 117 mg/dL, creatinine 3.29, and USG 1.015), mild hypercalcemia (11.5 mg/dL), and moderate hyperproteinemia characterized by moderate hyperglobulinemia (5.4 mg/dL). The abdominal ultrasound revealed mild left pyelectasia (accumulation of urine within the renal pelvis, likely due to polyuria, renal insufficiency, or pyelonephritis), mild splenic changes (likely caused by lymphoid hyperplasia or extramedullary hematopoiesis), and reactive submucosal lymphoid follicles in the colon. A rectal scrape was submitted for cytologic evaluation. It revealed neutrophilic colitis with evidence of protothecosis infection. On cytology, Prototheca sp. varies from 5-10 x 10-20 μ m and has a colorless wall and granular dark blue content. They may contain two to numerous endospores (see pictures). Additional findings included evidence of acute and chronic hemorrhage, undigested material (e.g., fat), mucus, and overgrowth of spore-forming bacteria. Given the renal azotemia and presence of these organisms in the rectal scrape, the urine was also submitted to cytology, where rare organisms with similar morphology were found. The presence of the organism was confirmed after positive fungal culture.

Prototheca sp. is a unicellular algae organism ubiquitous in the environment. It rarely infects dogs via inhalation or ingestion. However, when it does, it primarily invades the gastrointestinal, central nervous system, and eyes. Rarely, kidneys, muscles, and liver can also be affected. In cats, there is a cutaneous form of protothecosis. Immunosuppression and genetic predisposition may play a role in the pathogenesis. Unfortunately, the prognosis is poor, and dogs do not or only mildly respond to treatment with antifungal drugs, with complete remission seldom achieved. The dog was discharged with systemic voriconazole treatment. Approximately 10 days later, the owner said the dog was more active, had a good appetite, and had no more loose stools. We have no current follow-up.

Priscila B. S. Serpa, DVM, MSc, DSc, DACVP (Clinical), Virginia Tech



Endospores of Prototheca spp.

Biliary adenocarcinoma in a bearded dragon

An approximately 10-year-old, female bearded dragon presented to the referring veterinarian with a several-month history of weight loss and lethargy. The patient was moribund on presentation and a mass was palpable in the mid-abdomen. The owner elected euthanasia and requested a necropsy. External examination revealed poor body condition. The abdominal mass palpated by the rDVM was a severely distended gallbladder. In the liver were approximately a dozen, firm, tan masses measuring up to 1 cm in diameter. Histologically, the nodules were composed of anaplastic epithelial cells forming tubules. Other findings in the liver included multiple bile ducts in each portal triad, often surrounded by concentric layers of fibroblasts and connective tissue. The coelomic fat pads contained amphophilic fibrillar material (serous atrophy of fat) and two nodules of neoplastic cells similar to those in the liver. The diagnosis was biliary adenocarcinoma with metastases to the coelomic fat pads. The enlarged

gallbladder and serous atrophy of fat were most likely due to prolonged anorexia. Neoplasia was historically considered unusual in reptiles, but recent studies have shown that captive lizards, especially bearded dragons and panther chameleons, have a relatively high rate of neoplastic lesions. Skin tumors are the most commonly diagnosed neoplasms in pet lizards (probably due to ease of detection by the owner), but a variety of other tumors (including biliary adenocarcinoma) have also been reported.

Teresa Southard DVM, PhD, DACVP. Virginia Tech

LABORATORY NEWS



ViTALS

Roger Ramirez-Barrios was awarded the 2023 Zoetis Research Award for his work on *Echinococcus multilocularis* and *Trypanosoma cruzi*, parasites that cause disease in humans and animals. The award recognizes research contributions that advance veterinary medicine. We are so lucky to have such an amazing parasitologist on our team!

Priscila Serpa, Brie Trusiano, Christina Pacholec, and Marlie Nightengale showcased their work at the annual ACVP and ASVCP meeting in Chicago, mentoring undergraduate and graduate students, and participating in the Veterinary Students and Residents forum. We appreciate their mentoring and recruiting efforts, and showcasing the work that ViTALS does!

Our clinical microbiologist, Tessa LeCuyer, made the decision to move closer to her family on the west coast, and accepted a position at UC Davis. Her contributions to ViTALS, from overseeing the standing up NAHLN and CWD testing, expanding the serology section, and helping the bacteriology section heads improve procedures, have been invaluable to our clients, and our state and federal partners. Her shoes will be big ones to fill!

Tanya LeRoith DVM, PhD, DACVP. ViTALS Director

VDACS

VDACS has signed a contract with Gauthier Alvarado Associates (GAA) to provide architectural and engineering services for expansion and renovations at the Warrenton, Harrisonburg, and Lynchburg Regional Animal Health Laboratories. This capital project was approved by the General Assembly in 2022 and will encompass approximately 8,800 square feet of new space across the three facilities. The expansions and renovations will allow for more efficient lab processes and accommodate new testing methodologies that promote the agency's core mission of supporting Virginia agriculture. The design process will occur over approximately the next year, with an expectation of a year construction time. James Madison University is providing procurement, project management, and inspection services to VDACS for this capital project.

The Office of Laboratory Services has performed an in-depth data analysis for determining appropriate fees for services and remains dedicated to maintaining the most up to date fee schedule on our VDACS website: https://www.vdacs.virginia.gov/animals-animal-healthlaboratory-services.shtml. The fee schedule includes locations for testing, up to date pricing, and preferred specimen type. Fee increases for 2024 are not a standard percentage but instead factor in supply and testing costs in addition to subsidy and goals to maintain affordable pricing for agricultural producers. New fees went into effect on January 1, 2024.

Testing platforms are continuing to evolve based on the needs of our clients! Our Ruminant Abortion and Bovine Respiratory PCR panels in Lynchburg are up and running, and now can include BVD PCR testing. Samples for these tests range from fluid to swabs to tissue. In Harrisonburg, poultry necropsy, tissue culture, and emergency PCR testing markedly increased in both December and January due to an avian metapneumovirus outbreak, keeping multiple departments busy. Currently the molecular and serology department are

are working to add both Avian Metapneumovirus (aMPV) serology and PCR testing to our diagnostic testing options. In Warrenton, analysts are working on bringing in CL SHI testing capabilities and are continuing to maintain a heavy necropsy and CEM culture load. Wytheville is gearing up for annual fish testing, covering both diagnostic and regulatory testing for fish health in the Commonwealth and surrounding states.

Lots of staffing changes!

- A new bacteriologist started at the Warrenton Laboratory on November 25th! We are excited to have Hayley Crossman join our team in that location.
- We had an internal promotion to the new Microbiologist Supervisor in Lynchburg as of December 10th. Congratulations to Kara Harrison!
- New regional quality coordinators started on December 10th (Sarah Mayer) and January 10th (Keandra Walthall) based out of Harrisonburg and Lynchburg, respectively. We are excited to have them on board and to continue to build on our quality program!
- A new molecular microbiologist was hired in Lynchburg and started February 12th. Welcome to Bethany Latwinski!
- The Harrisonburg administrative assistant retired on January 1 with 53 years of state service. Her replacement, Michelle Reedy, will be starting with us on February 25th!

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.

Looking forward to a great 2024!

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

LABORATORY LOCATIONS

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