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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

Johne's Disease

Johne's disease, also known as "intestinal mycobacteriosis" or "paratuberculosis", is a chronic inflammatory condition that affects many species, most notably cattle. Johne's disease is a large fiscal and population detriment to the food animal industry, with loss of over $198 million in the United States dairy cattle industry. The prevalence in the United States is approximately 68% in dairy herds, 12% in beef herds, and up to 10% in sheep flocks.

What Is the cause?

The causative agent, Mycobacterium avium spp. paratuberculosis, is a slow-growing intracellular bacterium. Most animals are exposed and infected with the bacteria at an early age, usually within the first 6 months of life. The bacteria can be transmitted through feces, milk, colostrum, and in utero. In large ruminants, it causes inflammation of the intestinal wall, leading to decreased absorption of necessary nutrients, and inciting diarrhea. The bacterium also can transmit to other animals, including small ruminants (sheep and goats), poultry, pets such as dogs and cats, and has been loosely associated with Crohn's disease in humans.

What Are the Clinical Signs?

Animals are usually within the range of 2-6 years old when they begin to exhibit clinical signs. The most notable clinical sign of Johne's disease is weight loss with mantaining normal appetite. Other clinical signs can include diarrhea, decreased milk production, and in extreme cases, bottle jaw (edema within the submandibular region). Small ruminants are notorious for having undetectable disease in the majority of cases. The USDA has defined four stages of Johne's disease:

Stage 1: Silent, undetectable infection. Younger animals are typically within this category. These animals are recently infected and do not display any clinical signs. Diagnostic testing will result in a negative or inconclusive result.

Stage 2: Subclinical infection. These animals are not displaying clinical signs, but have variable results.

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Stage 3: Clinical manifestation. Advanced infection is presumed at this point, and animals begin to display clinical signs. These animals will proceed to stage 4 of the disease within a short period.
Stage 4: Animals at this stage are extremely thin (body score <2/9 for beef cattle, 1/5 for small ruminants), and have bottle jaw (fluid accumulation within the jaw area). If sent to slaughter, these animals are terminal and could potentially be condemned due to having systemic disease.

How Can We Test for Johne's?
The bacterium responsible for Johne's disease (Mycobacterium avium ssp. paratuberculosis) can be tested in numerous ways. The oldest and most traditional method of diagnosing Johne's disease is a fecal culture, utilizing Herrold's egg yolk agar (as also known as HEYA), which provides an excellent growing medium for optimal growth. However, histopathology and cytology of intestinal and liver tissue samples have also been utilized earlier than other available diagnostic tests. Notable findings on histology include expansion of the lamina propria of the ileum mucosa with foamy macrophages and multinucleated cells, crypt hyperplasia and blunting of villi, as well as granulomatous inflammation in mesenteric lymph nodes. Histopathology has also become a more reliable source of diagnosis among small ruminants, due to the variability among other tests and the lesions they possess. Utilizing acid fast staining on histology or cytology, Mycobacterium avium ssp. paratuberculosis can be identified in tissues if captured, due to its capsular biology that does not allow more common stains such as Wright-Giemsa and Diff-Quick to detect it. More recently, other diagnostic modalities such as ELISA, AGID, CFTs, and PCR have been developed and became available.

What Can Be Done to Prevent Johne's disease?
Due to the herdwide effects of the bacteria, it is imperative to be able to identify the animal(s) with clinical signs and subsequently remove them from the herd to decrease the likelihood of shedding. Depending on the state, acquiring a veterinarian that is a Johne's disease Certified Veterinarian may be necessary, as they provide the most up-to-date and accurate information regarding diagnosing, monitoring, and controlling the disease. The USDA has also created a uniform program standard for a Johne's disease outbreak. The most crucial components of controlling and reducing the incidence of Johne's disease is adequate and prompt diagnostic testing of suspicious animals and animals that cohabitate with them. Proper environmental and colostrum management of neonates and youngstock should be considered a top priority to reduce infection chances. Other protocols include buying from herds that are certified as having no presence of Johne's disease.

There have also been strides in producing newer diagnostic testing for producers at home, although these are currently theoretical and experimental. For example, interferon-gamma testing has been present in diagnostic laboratories since the 1980s, which utilizes this specific cytokine to detect the presence of mycobacterial antigenic response within T-lymphocytes. However, there is high variability within this specific test in terms of cross-reactivity and significant false positive/negative rates. Therefore, it is likely that further research should be implemented to use this technology efficaciously to detect and monitor Johne's disease. Another potential candidate for future testing methods is genomic testing. This technology includes sequencing and biosensors for the rapid detection of Mycobacterium among individual animals. The sequencing in most cases was pointed towards the IS900 target biomarker, which has been shown to provide a more accurate result of detection in preliminary studies as opposed to other candidates of potential biomarkers. This, in turn, will provide producers with an on-farm test that will potentially provide both accurate and fast results.

Producers, veterinarians, and public health officials are becoming increasingly aware of Johne's disease. To better understand how the bacterium works and manifests, we have been able to develop and modify our diagnostic testing methods to provide reliable results. It is imperative to stay at the forefront of this disease to reduce the likelihood of herd-level threats and the potential spread among other species and even into humans. It is essential to consider many factors when identifying what diagnostic tests should be utilized, as the many options may provide better benefits in different scenarios when compared to one another. Speaking with a local Johne's disease-certified veterinarian, as well as utilizing veterinary pathologists and epidemiologists found at diagnostic or state laboratories, may prove beneficial in identifying which diseases and tests should be considered.

Andrew Arnold, Student of Veterinary Medicine (class of 2022), Virginia Tech.

RUMINANTS

Rabies in a steer

An 8-month-old steer presented to the Virginia-Maryland College of Veterinary Medicine necropsy service following a short history of abnormally friendly behavior. Oral mineral and vitamin supplements had been administered by the producer, who wore gloves; there was no change in behavior. A skunk had been seen in close proximity to the animal several weeks prior. There were no gross findings on necropsy, but Negri bodies were seen in neurons histologically, and rabies was confirmed by the Virginia Department of Health. This is an important reminder that rabies is endemic in Virginia, and any unvaccinated or undervaccinated animal exhibiting any change in behavior may be infected. Rabies is transmitted through saliva, most typically through bite wounds, although infected saliva coming into contact with cuts on the hands or with exposed mucous membranes can transmit this deadly virus to human beings. Do not approach or pet wild or feral animals that are behaving oddly, even if they seem to be acting docile. If you are bitten by a wild or a feral animal, wash the wound thoroughly and contact your primary care physician for advice. Rabies is effectively prevented by post-exposure vaccination.

Vanessa Oakes DVM, DACVP, Virginia Tech.

Dicoumarol intoxication in a cow

An adult beef cow was submitted to the Wytheville Regional Lab for post-mortem examination. The owner reported the loss of eight cows over a three-week period. Many of these animals had exhibited abrupt weight loss and hemorrhage from body orifices. Anthrax was therefore one of their concerns. Necropsy of the cow revealed mild hemorrhage from the vulva and anus. No overt hemorrhage was evident internally. There were no significant bacteriological findings and histopathology revealed only mild inflammation in multiple organs. Spleen was forwarded to the Texas Veterinary Medical Diagnostic Laboratory for anthrax culture. Results were negative. Liver was submitted to the Pennsylvania Animal Diagnostic Laboratory for an anticoagulant screen. Results were positive for dicoumarol. Dicoumarol is an anticoagulant substance found in certain forages and plants. Molds are typically involved as dicoumarol is generally detected in spoiled forages. Sweet vernal grass, lespedeza, and sweet clover are three potential plants commonly implicated. Poisoning is dose dependent and a delay of 2 to 14 days often occurs between the feeding of the dicoumarol containing hay and the appearance of the hemorrhagic syndrome. Clinical symptoms can be non-specific and vague, and are generally those associated with anemia (weakness, exercise intolerance, collapse, rapid breathing). Sudden fatal hemorrhagic diathesis may be the sole premonitory sign. Parenteral vitamin K therapy is warranted.

Christopher Halsey DVM, RAHL Wytheville.

Ruminal foreign body and hardware disease in a 3-month-old beef calf

Three 3-month-old calves from a group of 11 died, and two were submitted to VMCVM for necropsy. Clinical signs prior to death were minimal and included increased salivation in one calf and low body temperature in another. Both of the calves submitted for necropsy were in poor body condition and had large, tangled pieces of rope in their rumens. In addition, the calf with a history of increased salvation had a 3 cm long, J-shaped piece of wire in the reticulum, with the straight end extending through the esophageal wall at the level of the diaphragm and penetrating the accessory lobe of the lung. At the site of penetration, the lung lobe was expanded by a thin-walled, 2.5 cm diameter abscess, Smaller abscesses were scattered throughout both lung lobes. In both calves, the ruminal foreign body likely contributed to decreased feed intake, poor body condition, and debilitation. The calf with the wire foreign body also had compromised respiratory function. Salivation in this calf was most likely secondary to esophageal inflammation associated with foreign body perforation.

Cattle are indiscriminate eaters and consumption of foreign material is common. Hardware disease, where an ingested wire foreign body penetrates the gastrointestinal tract, is most common in mature dairy cattle. Magnets are frequently used to attract metal

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foreign bodies in the reticulum to prevent hardware disease, but magnets are typically not given until the animal is about 6 months old. In adult cattle, hardware disease often manifests as traumatic reticulopericarditis, where the wire penetrates through the wall of the rumen and through the diaphragm into the pericardial space, resulting in fibrinous pericarditis. Reticuloperitonitis, characterized by penetration of the wire through the forestomach wall into the peritoneal space, has also been reported. Damage to the lung, in this case, may be a consequence of the differences in the relative size and position of the forestomachs in a calf compared to a mature cow.

Teresa Southard DVM, PhD, DACVP, Virginia Tech.

HORSES

Aortic rupture in a horse

A 2-year-old Friesian mare presented to the Virginia-Maryland College of Veterinary Medicine necropsy service after being found deceased in the pasture one morning. This animal was fully up to date on vaccines, was healthy and in good body condition, and the owner reported that there was no indication of disease or discomfort prior to death. An approximately 4 cm in length, full-thickness perforation was within the aorta, and the surrounding soft tissue was markedly expanded by hemorrhage. Aortic rupture is an uncommon cause of sudden death in horses, with a poorly elucidated etiology. Friesian horses are overrepresented, and there is evidence that an underlying genetic defect impacting collagen formation may be the cause.

Vanessa Oakes DVM, DACVP, Virginia Tech.

An unusual case of recurrent choke in a horse

An 18-year-old, mixed breed mare was evaluated at the VTH for a second episode of choke within 2 months. The horse was treated with fluid therapy, non-steroidal anti-inflammatories, and antibiotics for pain and secondary aspiration pneumonia, and then sedated for endoscopy. Before the endoscopy could be performed, the horse began bleeding profusely from the nose and mouth and was euthanized and submitted for necropsy. Gross examination revealed impacted ingesta in the thoracic esophagus with circumferential necrosis of the corresponding esophageal wall and a focal, full-thickness defect. The arch of the aorta and the proximal descending aorta were severely dilated, the aortic wall was thickened, and the intimal surface roughened. At the level of the esophageal impaction there was a defect in the intima of the aorta communicating with a large intramural hematoma, which also communicated with the lumen of the esophagus through the site of rupture. These findings were interpreted as an aortic aneurysm with dissection and esophageal compression, leading to recurrent episodes of choke. Ischemic necrosis of the esophageal wall, combined with increased pressure in the hematoma, likely caused rupture of the esophageal wall and the remaining layers of the aortic wall, forming a direct communication between the aorta and the esophagus. Aortic aneurysms and dissections are rare in horses. Potential causes include Strongylus vulgaris migration, bacterial or fungal aortitis, collagen vascular diseases, and nutritional imbalances such as copper deficiency. The cause was not determined in this case.

Teresa Southard DVM, PhD, DACVP, Virginia Tech.

COMPANION ANIMALS

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Proliferative pleuritis in a foxhound

A 2-year-old, intact, female foxhound was submitted for respiratory difficulty and fluid in the thoracic cavity. At necropsy, the thoracic cavity contained abundant red-brown opaque fluid. Extending from the pleural surfaces of the thoracic wall, pericardial sac, and lungs were multilobulated, gelatinous, dark red masses (proliferative pleuritis). The lungs were gray, collapsed and rarely adhered to the thoracic wall. A common cause of proliferative pleuritis in dogs is an Actinomyces or Nocardia infection. Nocardia and Actinomyces are filamentous bacteria that can be found in the environment (soil, dust, plant material, etc.) or oral cavity, GI tract, and genitals, respectively. Infection is often associated with a migrating plant awn, puncture wound, or inhalation and is commonly seen in foxhounds and other hunting breeds. Both bacterial species can be difficult to culture. In this case, there were filamentous acid-fast bacteria that were suggestive of Nocardia species on impression smear. A possible differential for this finding is a mesothelioma which is rarely seen in dogs and may be associated with exposure to dust and chemicals (asbestos, iron, or silica).

Jaime Weisman DVM, MS, RAHL Warrenton.

Acute leukemia in a cat

A 10-year-old, female, American Shorthair cat presented to the VMRCVM Teaching Hospital due to lethargy, inappropriate urination, and acute onset of dyspnea. Her pulse was weak and there was increased respiratory rate and effort, despite normal lung sounds bilaterally. She was euhydrated, with pale mucous membranes, and dull. A complete blood count was performed and a severe nonregenerative normocytic normochromic anemia (hematocrit 9.6%), moderate thrombocytopenia, and mild leukocytosis were identified. The automated hematological counter indicated the presence of a prominent monocytosis. However, on pathological review of the smear, 35% of the leukocytes were identified as blasts, not as monocytes, indicating an acute leukemia, highlighting the importance of blood smear evaluations. In addition, the morphology of these blasts (cells with features of immaturity, such as open chromatin and presence of prominent nucleolus) was consistent with erythroid precursors, and a presumptive diagnosis of erythroleukemia was established.

Approximately 5% of cats in the United States are infected with FeLV or FIV, and these viruses can predispose to bone marrow suppression, cancer (e.g., lymphoma and leukemia), and chronic inflammatory conditions. Although FeLV/FIV infections are the most common causes of erythroleukemia in cats, this patient was negative for the FeLV antigen-FIV antibody rapid test. However, in approximately 10% of FeLV infections, the cat's immune response can eliminate the virus from the blood but is not vigorous enough to completely eliminate it from the body (also known as regressive infections), and PCR tests in the bone marrow of the patient may be necessary to confirm the diagnosis.

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

Histoplasmosis in a cat

A 1-year-old, male castrated Domestic Shorthair cat presented for a one-week history of lethargy, decreased appetite and pain when walking. On physical exam the patient was febrile (103.7 F) and painful on thoracolumbar and carpal/tarsal joint palpation. Bloodwork showed a mild nonregenerative anemia, a hyperproteinemia characterized by a mild hyperglobulinemia and hypoalbuminemia and a mild hyperbilirubinemia. Joint fluid was collected from the tarsi and carpi for cytologic evaluation. The samples were of high cellularity with many foamy activated macrophages, nondegenerate neutrophils and fewer small lymphocytes. The macrophages frequently contained 2-4 um round to ovoid yeast with a 1-2 um basophilic center and a 1 um colorless halo (see image). The yeast occasionally displayed narrow-based budding. The cytologic interpretation was pyogranulomatous inflammation secondary to fungal disease (Histoplasma capsulatum infection). Histoplasma is ubiquitous in the environment and frequently found in soil (especially where birds are housed). The most common mode of transition is ingestion or inhalation from the contaminated environment and subsequent infections can spread widely through the body. Multiple organs may be involved and can include the gastrointestinal tract, bone marrow, liver, spleen, lymph nodes, eyes, among others.

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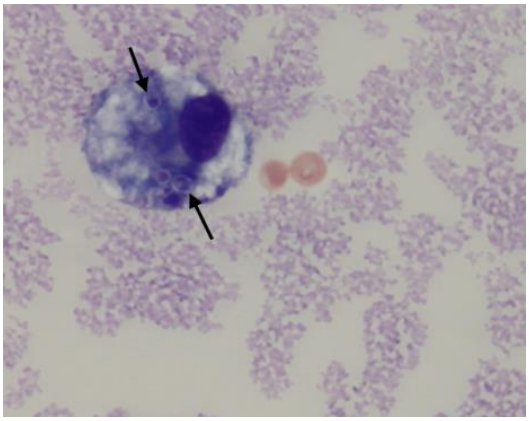
The yeast replicate in the macrophages and are released upon cellular death, allowing them to continue to spread. Anti-fungal therapy (e.g. itraconazole) can be curative and monitoring response to therapy with a urine histoplasma/blastomycosis antigen test is helpful.

Christina Pacholec, DVM, Clinical Pathology resident and master's candidate (2023) and Natalia Strandberg, DVM, MS, DACVP (Clinical), Virginia Tech.



Synovial fluid from the tarsus, 100x oil, modified Wright stain. A single macrophage with intracellular yeast (black arrows)

Pseudocarcinomatous biliary hyperplasia in a Bearded Dragon

A 6-year-old, female Bearded Dragon with a history of inappetence, lethargy, and recent change in the diet was received for necropsy. Gross examination revealed celomic and pericardial effusion and a dark brown and rubbery liver. Remarkable histologic findings are seen in the liver, where almost 90% of the liver parenchyma has been replaced by numerous tubules conformed by a single row of cuboidal cells supported by an abundant fibrovascular stroma, resembling biliary epithelium. Additionally, choleliths are seen in the gall bladder and scattered bile ducts. The diagnosis is consistent with pseudocarcinomatous biliary hyperplasia. Biliary hyperplasia is an unspecific response to chronic insults in the liver and bile duct. The presence of choleliths suggests biliary obstruction as the cause and has been reported as a common problem in Bearded Dragons under high protein and high-fat diets. Additionally, the severity and extension of the changes are similar to those reported in iguanas as psudocarcinomatous biliary hyperplasia, which can also occur due to similar circumstances.

Valentina Stevenson DVM, Virginia Tech.

LABORATORY NEWS

ViTALS

We are currently preparing for our AAVLD accreditation site visit which is scheduled to take place this summer. This process will give us an external evaluation of our quality system and our goals of providing high quality diagnostic testing and continuous improvement of our processes. We are excited to host the accreditation team and show them what we've been doing over the last 5 years.

Kevin Lahmers spoke with AABP Executive Director Fred Gingrich about Theileria orientalis and the Asian Longhorn Tick. The podcast can be found here https://www.buzzsprout.com/814177/9084815-theileria-orientalis-and-the-asian-longhorn-tick. Dr. Lahmers is also the newest member of the AAVLD accreditation committee, and will conduct his first site visit as a member of the committee in the fall.

Lauren Lytle joined ViTALS on January 3rd in the clinical pathology laboratory. Lauren did her laboratory internship at University of Mississippi/Singing River Health System. Lauren has experience as a zookeeper and trainer (birds) but found her niche by working at Antech as a hematology tech.

Tanya LeRoith DVM, PhD, DACVP, ViTALS Director.

VDACS

The VDACS Office of Laboratory Services has been extremely busy!

New Microbiologist Supervisors have been hired in both the Warrenton and Lynchburg Laboratories. Welcome to the RAHL Warrenton Dr. Nabin Rayamajhi!

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and welcome to the RAHL Lynchburg Mr. Marcos Alejos! They are responsible for on-site management of testing analysts, and also serve in a backup capacity for testing. Our employees are preparing to be able to handle any outbreak needs, and continually cross-training in multiple disciplines to ensure continuity of services.

New tests are coming to the laboratory system, and are in the process of verification to ensure they are working properly in our labs. These tests include: antimicrobial residue testing for urine and serum (KIS test), a bovine respiratory PCR panel for common respiratory pathogens, a ruminant abortion PCR panel for common abortion pathogens, a Salmonella PCR. Our MALDI-TOF bacterial identification system is up and running and will hopefully shorten the process of identifying disease agents. We are continuously analyzing the needs of our clients and evaluating new tests to serve these needs.

Testing is continuing to be consolidated in order to shorten turnaround times, and analysis of the laboratory system is being completed by both internal and external evaluation bodies. Our employees are preparing to be able to handle any outbreak needs, and continually cross-training in multiple disciplines to ensure continuity of services.

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

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