Notes from Our Director...

- Thank you to everyone that took the time to participate in our user survey! We are currently evaluating the responses and comments and we will utilize the valuable information we received to ensure we are meeting your expectations and needs.

- Please be sure that your livestock and companion animals are up to date with their vaccination status (and veterinary care). Summer is traditionally peak rabies season, and this year North Carolina has already had 7 reported cases of rabies in livestock. Also, mosquito born disease season is here! It is not too late to vaccinate your horses for mosquito born viral encephalitides (Eastern Equine Encephalitis, Western Equine Encephalitis, West Nile Virus, Venezuelan Equine Encephalitis). These fatal diseases are preventable by appropriate vaccination!

Now is the time to vaccinate your equine against mosquito-borne diseases and rabies.
• The Rollins Animal Disease Diagnostic Laboratory has been accepted as a Tier 1 laboratory in the Veterinary Laboratory Investigation and Response Network (Vet-LIRN). This program coordinates facilities, equipment, and professional expertise of government and veterinary diagnostic laboratories across the country and Canada to respond to high priority chemical and microbial feed/drug contamination events. The network provides the means for rapid response to reports of animal injury and establishes protocols to facilitate veterinary diagnostic reporting to the FDA.

• The spread of African swine fever (ASF) continues its spread through Asia. First reported in northeastern China in August 2018, the highly contagious, often fatal pig disease quickly swept through the country, causing the death or culling of more than 1 million pigs. It does not affect human health and cannot be transmitted from pigs to humans. In recent weeks, it has jumped borders to Vietnam, Cambodia, Mongolia, Hong Kong, and possibly North Korea. Animal health experts agree that the disease will inevitably spread farther.

• Our laboratory system continues to work closely with North Carolina’s Department of Agriculture, USDA APHIS, the swine industry, and practitioners to prepare to respond to an ASF detection or outbreak within the United States. As a Level 1 NAHLN lab, the Rollins laboratory plays an important role in disease outbreak detection and response. The Rollins laboratory has multiple staff members that have successfully completing ASFV PCR Proficiency Tests provided by USDA NVSL. This ensures increased capacity and rapid response for ASF testing. In February, NCVDLS joined state and federal animal health officials as well as swine producers in an ASF exercise held in Goldsboro, North Carolina.

• Beginning June 1, 2019 the U.S. Department of Agriculture (USDA) is furthering its overall African Swine Fever (ASF) preparedness efforts with the implementation of a surveillance plan. As part of this plan, the Animal and Plant Health Inspection Service (APHIS) will work with the swine industry, the states, and veterinary diagnostic laboratories to test for ASF. To make this program as effective and efficient as possible, USDA will add ASF testing to our existing classical swine fever surveillance. We will test samples from the same high-risk animals, using the same overall process, but will test for both diseases instead of one.

Jim Trybus, DVM, DAVCP
Welcome Dr. Anil Thachil!

We are pleased to announce that Dr. Anil Thachil has joined our laboratory as our Bacteriology Section Head!

Dr. Anil Thachil is a Veterinary Clinical Microbiologist joining the Rollins Laboratory, Raleigh, NC. Dr. Thachil worked at Cornell University Animal Health Diagnostic Center / New York state Veterinary Diagnostic Laboratory (NYSVDL), Ithaca, NY for the last five years, where he was the Director for the Bacteriology & Mycology lab. As Director, he oversaw routine diagnostic activities in the Bacteriology lab along with implementation of new tests, proficiency testing for high consequential pathogens, and supervised laboratory personnel that conduct various molecular, serology and conventional diagnostic tests, serving a diverse clientele from all over US. He was actively involved in multiple research projects as Principal Investigator or Co-investigator, quality assurance, and safety programs, client consultations, budgeting and fiscal management of his section.

Dr. Thachil earned his professional degree in Veterinary Medicine (BVSc) from the Kerala Agricultural University in India before he received advanced training in Veterinary Clinical Medicine and Veterinary Bacteriology. Dr. Thachil earned a PhD in Veterinary Bacteriology (Veterinary Infectious Diseases) from the University of Minnesota, Twin Cities, MN. His PhD thesis was focused on pathogenesis and prevention of Clostridial dermatitis in turkeys. He then moved to Iowa Veterinary diagnostic lab where he did a post-doctoral research training at Iowa state university, Ames, IA for a short time before he moved to Cornell. Dr. Thachil is board certified in Veterinary Bacteriology and Virology by the American College of Veterinary Microbiologists (ACVM) since 2012.

Dr. Thachil has already made significant contributions to control emerging diseases through development of vaccines and diagnostic assays. Dr. Thachil developed safe and effective *C. perfringens* and *C. septicum* vaccines to prevent mortality due to Clostridial dermatitis in turkeys in affected poultry farms. Dr. Thachil was also instrumental in developing indirect ELISA assays for detecting *Clostridium perfringens* and *C. septicum* infection in turkeys. Dr. Thachil's expertise in diagnostic assay development was once again useful in conducting serological surveillance testing for recently emerged porcine deltacoronavirus infection in pigs in the US. He used recombinant derived fusion proteins to detect IgG antibodies by indirect enzyme-linked immunosorbent assay (ELISA) testing for porcine deltacoronavirus infection in pigs.

In addition to his veterinary diagnostic interests, he is also interested in teaching and research. His current diagnostic and research interests are focused on the development and evaluation of novel
outer membrane vesicle vaccine for clostridial dermatitis. Dr. Thachil have had experience working with FDA and USDA projects to address the issue of antibiotic resistance in food and companion animals. He is actively associated with professional associations such as CLSI, American Association of Veterinary Laboratory Diagnosticians and American College of Veterinary Microbiologists. He has authored or co-authored various publications in peer-reviewed journals, and is an ad-hoc reviewer for several journals including Avian diseases, PLOS-one, Journal of Clinical Microbiology, and Journal of Veterinary Diagnostic Investigation.

Dr. Thachil is married to Toncy Edakalathoor, a mathematician, and has one daughter Anlin. They enjoy outdoors and like traveling and cooking. They are very excited about moving to the Raleigh area, which will provide them with great opportunities for career, educational and recreational activities.
A 408kg, 6-year-old, female, black, Angus beef cow in very thin body condition with mild to moderate postmortem autolysis with a 10-day history of weight loss and inappetence was submitted to the laboratory for necropsy on 4/30/2019. On the day prior to death, the animal became recumbent and was taken to the veterinarian for evaluation. The veterinarian suspected pneumonia based on auscultation of the chest cavity and treated the cow accordingly. The cow was taken back home however it never stood up and was found deceased the following morning. This is the second adult cow with this clinical history to die in the past month. On examination, the spinal column and pelvis are prominent and a small amount of subcutaneous and visceral adipose tissue was evident throughout the body. The adipose tissue around the heart was partially replaced by yellow gelatinous material. The bone marrow was reddish white and gelatinous. Throughout the carcass, the peripheral and visceral lymph nodes were enlarged and lymph node chains were prominent in the inguinal region, submandibular region, and along the trachea/esophagus. The largest lymph node along the thoracic trachea measured 23cm x 3.5cm x 1.5cm and the largest inguinal lymph node measured 10cm x 4cm x 3.5cm. The wall of the abomasum adjacent to the reticulum was markedly expanded by translucent, gelatinous material. The spleen was diffusely enlarged with a meaty consistency. The liver was markedly enlarged measuring 85cm x 55cm x10-12 cm (at the thickest point). The liver was mottled pale tan and red and an enhanced lobular pattern was evident throughout the liver on cut section. The lungs were diffusely heavy and wet and pale tan, frothy fluid was present throughout the trachea and airways. A few, 2-5mm diameter, non-raised, pale tan foci were observed in the renal cortex and medulla.

Figure 1. Pharyngeal/cervical lymph nodes. Prominent lymph node chain observed in the laryngopharynx and along the trachea
Figure 2. Liver. Diffuse enlargement of the liver and pallor due to invasion of neoplastic lymphocytes

Figure 3. Mesenteric lymph nodes. Lymph nodes are enlarged and prominent
On histopathological examination, lymphoma was found in the all the lymph nodes, uterus, epicardial adipose tissues, liver, kidney, spleen, abomasum, and bone marrow. Additional lesions observed include: centrilobular necrosis was in the liver, pulmonary edema, and fibrinohemorrhagic and necrosuppurative abomasitis with granulation tissue.

Serum from the animal tested positive on ELISA for bovine leukemia virus (BLV) antibodies.

There are two types of bovine lymphoma: a sporadic type not associated with viral disease and enzootic bovine leukosis which is associated with BLV. The distribution of lesions varies depending on the type of lymphoma present. Non-BLV lymphoma (sporadic form) is divided into three forms: thymic, juvenile multicentric, and cutaneous. Bovine thymic lymphoma is most common in beef cattle ranging in age from 6 to 24 months of age. It commonly causes presternal swelling, jugular distention, localized edema, and compression of the esophagus. Respiratory distress may ensue due to displacement of the lungs by a large mediastinal mass. The juvenile multicentric type most commonly occurs in calves between 3 and 6 months of age but may be present at birth. It is characterized by widespread symmetric lymph node enlargement, leukemia, and lesions in the liver, spleen, kidney, and sometimes skeletal muscle. The cutaneous type is the least common form and occurs most commonly in cows between 2 and 3 years of age. Numerous plaque-like, round, raised lesions are observed on the head, sides, and perineum and the lesions wax and wane over a period of months. Ultimately neoplasia spreads to the visceral organs and the form becomes indistinguishable from those of BLV-associated lymphoma.

In adult cows, lymphoma is mainly the enzootic bovine leukosis form caused by infection with BLV. BLV is an oncogenic retrovirus that infects lymphocytes (white blood cells). Proliferation of these virus-infected cells can lead to a persistent lymphocytosis which is the benign form of disease or develop into neoplastic cells that invade into many different organ systems and develop tumors (lymphoma). Approximately two-thirds of cows infected with BLV remain asymptomatic. Around 30% of cows infected with the virus will develop lymphocytosis and less than 5% of cows infected with BLV will actually develop lymphoma. Clinical signs associated with cancer are highly variable because they depend on which organ system is invaded by neoplastic lymphocytes. Clinical signs of infection can include: peripheral and/or internal lymphadenopathy, dyspnea (difficulty breathing), bloat, jugular vein distention, tachycardia, brisket edema, weight loss, decreased milk production, fever, inappetence, infertility, rear limb weakness/paralysis, exophthalmia, and increased lymphocyte counts in blood. With BLV-associated lymphoma, lymphoma is found in the peripheral, pelvic, and abdominal lymph nodes as well as the abdominal wall, kidney, right atrium, retrobulbar space, spinal cord, uterus, liver, and spleen. Bovine leukosis virus is transmitted horizontally through the transfer of blood and blood products that contain infected lymphocytes. BLV-infected lymphocytes can be transmitted through natural breeding, blood-sucking arthropods, or iatrogenic by use of contaminated needles, ear tagging, and dehorning equipment, and the use of whole blood vaccines. Disease is much more common in dairy cattle than in beef cattle. Infection is lifelong and characterized by the development of circulating antibodies.

As there is no vaccine available for BLV, management practices must be relied upon to eradicate BLV from the herd. AGID and ELISA can be used to detect antibodies in serum, and PCR can be used to detect the virus in blood, milk, semen, and tissue samples.
A 9 day old Wheaton terrier puppy was submitted for a necropsy. The puppy was the fifth puppy out of a litter of nine to expire. The puppies showed signs of acute dyspnea before expiring.

The necropsy examination revealed a serosanguinous pleural effusion. The lungs were wet and heavy. The lung tissue samples sank when placed in formalin. Petechiae were found on the liver and kidneys (figure 1). The petechiae were present on the both the capsule and cut surface of the kidneys. The liver was also congested with irregularly shaped 2 to 3 mm multifocal to coalescing areas of pallor. Histopathology of the kidneys showed renal tubular necrosis with intranuclear inclusion bodies. Hepatocellular necrosis with intranuclear inclusion bodies were found in the liver. Interstitial necrosis was present in the lung tissue.

Canine herpesvirus is an opportunistic virus that readily infects pregnant females in the last three weeks of gestation. It causes abortions, stillbirths, and a neonatal viremia in the first three weeks of life. Herpesvirus is an extremely common canine virus and most dogs with social contact with other dogs will have been exposed. When adult dogs are initially infected mild respiratory signs may be observed or may be so mild they go unnoticed.

Puppies may have abdominal pain/bloating, shallow breathing, hypothermia, constant vocalization, and weakness. Most puppies will expire within 24-48 hours of the onset of clinical signs.

Canine herpesvirus is spread by air and direct contact with nasal secretions. Once a dog is infected, it is infected for life. An infected dog may start shedding the virus during periods of stress. Once the mother has been exposed (as long as the first exposure is not within the last three weeks of gestation or the first three weeks after birth), pregnancies should not be affected. Mothers that have lost a litter due to herpesvirus are expected to have normal subsequent litters. However, a mother dog with prior exposure may be able to re-shed the virus if she is stressed and becomes immunocompromised.

During the last three weeks of gestation and the first three weeks after whelping, the mother and puppies should be kept quarantined from other dogs to prevent exposure to dogs who may be carrying the virus. Hands should be washed before handling the mother and puppies. Canine herpesvirus does not live long in the environment and is susceptible to common disinfectants.
Signalment: 8 month old female mix Canine

History and Gross examination:
Ulcerative and proliferative lesions on one front limb and one hind limb that have progressed in size. Antibiotics and corticosteroids show only minimal improvement with recurrence and worsening of lesions. Front leg was amputated due to large focally extensive lesions that were not amenable to removal and multiple lesions on rear limb were removed surgically.

Histopathology:
Section of haired skin and underlying subcutis is examined. The epidermis is elevated and the dermis and subcutis markedly expanded by a large multifocal and coalescing inflammatory infiltrates occasionally centered around hair follicles that are ruptured (furunculosis). The cells are composed of numerous degenerate and intact neutrophils admixed with moderate numbers of macrophages and smaller aggregates of eosinophils. The coalescing inflammatory aggregates form small cavitated spaces. Small bands of immature fibrous connective tissue and small, well differentiated blood vessels (granulation tissue) are located within the infiltrates.

Diagnosis:
Skin; dermatitis and furunculosis, pyogranulomatous and eosinophilic, severe, multifocal and coalescing, chronic
PAS stain showed no fungal organisms.
GMS stain showed small numbers of positively staining pseudohyphae

Mycology: Pure growth of *Pythium insidiosum*

Molecular: DNA sequence positive for *Pythium insidiosum*

Diagnosis: Cutaneous Pythiosis

Comment: *Pythium insidiosum* is a fungus-like, aquatic, opportunistic, flagellate oomycete with an affinity for warm stagnant water. Differ from true fungi in that they produce motile flagellae zoospores and cell walls that contain little to no chitin or ergosterol. Cutaneous disease is often seen on the extremities and muzzle region. Treatment of choice is surgical excision and amputation due to lack of response to anti-fungals. The systemic form of Pythiosis is often located in the proximal gastrointestinal tract and lymph nodes of younger large breed dogs.
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