Kansas State Veterinary
DIAGNOSTIC LABORATORY

MARCH 2016

DIAGNOSTIC *INSIGHTS*

Celebrating and Honoring the Life of

Gordon Andrews, DVM, PhD, Diplomate ACVP

October 13, 1953 - January 17, 2016

In this issue, we celebrate and honor our colleague and friend, Dr. Gordon Andrews, with a collection of his previous contributions.

On Sunday January 17, 2016, Gordon Andrews DVM, PhD, Diplomate ACVP, a senior pathologist in the Kansas State Veterinary Diagnostic Laboratory passed away at Stormont Vail Medical Center in Topeka, KS.

Gordon Andrews was born on October 13, 1953 in Batavia, New York. He received his bachelor's degree from Cornell University in 1971 and his doctorate of Veterinary Medicine from Oklahoma State University in 1984. He practiced in a general and emergency medicine practices in a New Jersey. In 1987 Gordon entered Kansas State University earning his doctorate in anatomical pathology in 1991, completing his veterinary pathology residency in 1992, and became a Diplomate of the American College of Veterinary Pathology the following year. He was a professor of diagnostic pathology in the KSU College of Veterinary Medicine for 22 years.

During those 22 years, he trained, taught, and mentored 27 pathology residents and hundreds of veterinary students.

Dr. Andrews' survivors include his wife Mary Anne and his two daughters, Katherine of Lawrence, KS and Emily of Manhattan. He is also survived by his parents Gordon and Barbara Andrews of Del City, Oklahoma.

Dr. Andrew's Residents

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In this Issue

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Lead Toxicity in Cattle: An Ongoing Environmental Problem

By Dr. Gordon Andrews *Originally published in the July 2014 issue.*

I recently received a 6-month-old calf for necropsy examination with a history of acute onset of neurological signs including blindness, recumbency, opisthotonus, paddling, and death within 12 hours of the onset of clinical signs. The submitter wanted to rule out rabies, and the rabies test was negative. There were no gross lesions in the brain or any other organ systems. The owner did not wish to pursue any additional diagnostic testing at that time. Within 2 days, 2 more calves from this group died with the exact same clinical signs and were submitted to me for complete necropsy examination to establish a definitive diagnosis. I was suspicious of lead toxicity, so collected whole blood from both calves for lead testing. Toxic levels of lead were present in the blood of both calves. I spoke with the owner about the diagnosis. He had also considered lead toxicity and had already searched the premises where the calves were held but had been unable to find any source of lead. Lead toxicity is the most commonly diagnosed toxicity in cattle at the diagnostic laboratory. Lead is found in many home, agricultural, and industrial products, and combined with cattle's curiosity, licking behavior, and indiscriminant eating habits makes lead toxicity common.

To order to protect the other cattle from possible lead poisoning, it is crucial to identify the lead source and remove it. However, finding the exact source of the lead exposure is not always straight forward. In cattle, common sources of lead are discarded auto / machinery batteries and improperly disposed of used motor oil. Other farm and ranch sources of lead include; grease, lead tire weights, linoleum, pipe fitting compound (pipe dope), shotgun pellets or lead bullets, lead-based painted wood, or those paint chips that have fallen off older barns or outbuildings.

In this case, the owner was unable to find any of the common sources of lead listed above, so submitted soil samples collected from the site of an old outbuilding which had burned down to the KSVDL Toxicology Laboratory. These soil samples contained very high levels of lead. Lead in soil can cause toxicity by direct ingestion of the soil or by inhalation of dust from the soil. Lead poisoning in animals should be considered a sentinel for possible environmental contamination by lead which can result in toxicity for other animals or humans, particularly children.

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Maximizing the Diagnostic Potential of Skin Biopsies

By Dr. Gordon Andrews

Originally published in the September 2015 issue.

For dermatological cases, properly obtained and interpreted skin biopsies can be the cornerstone of establishing a definitive diagnosis, or a differential diagnosis list that can be refined by additional diagnostic testing. To be successful, this requires effort by both the submitter and pathologist. The following guidelines are suggested to ensure that your pathologist has appropriate biopsies to examine and clinical information to interpret the biopsies and put the histological lesions into context to establish a diagnosis.

STOP medication if possible: When feasible; withhold the patient from drugs, particularly steroids, prior to obtaining biopsies. Steroids are anti-inflammatory and can alter the histologic lesions and nature of the inflammatory cell infiltrate.

DO NOT prep the biopsy sites: The pathologist needs to see the crust, scale, or exudate on the skin surface. Crust, scale or exudate on the surface may be the only change in some skin diseases, or may be part of a combination of lesions in other diseases. Etiological agents such as mites, yeast or bacteria may be in the surface crust. Pustules and vesicles are fragile and easily ruptured by prepping the skin.

ALWAYS use sharp dissection to obtain biopsies: Skin punch biopsy instruments are preferred. They are inexpensive, easy to use and take uniform clean tissue samples. They come in sizes from 2–8 mm diameter. Use the largest punch practical for the anatomic site. 8 mm is recommended for general use. Smaller biopsies may be needed for sensitive areas such as the nose, around the eyes, and feet. Small biopsy sites can be left open to heal. Larger sites can be closed with a single suture. If lesions have discrete borders, use a scalpel to take an elliptical biopsy with the long axis of the ellipse perpendicular to the edge of the biopsy. Never use laser or electrocautery to obtain skin biopsies. These instruments will burn tissue. Small skin biopsies can be completely burned and rendered useless for histopathology. Handle tissues gently. Crush artifact alters microscopic anatomy and destroys cellular detail. Label this biopsy as lesion border. Color differences that are grossly obvious can disappear when the tissue is fixed.

TAKE multiple biopsies: Multiple biopsies increase the probability of obtaining an accurate diagnosis. The diagnosis is seldom present in a single biopsy. Obtain biopsy samples from lesions that have differing gross appearances. Multiple biopsies may provide additional information about secondary problems like superficial pyoderma in addition to an immune mediated skin disease. In some cases (particularly diseases characterized by alopecia) a biopsy of clinically normal appearing skin can be helpful for comparison. Diagnostic microscopic lesions can be present in clinically normal appearing skin. Label this biopsy as normal appearing skin.

PUT the tissues in formalin as soon as possible: Desiccated tissue has altered staining characteristics and histologic appearance. Identify the anatomical location of each biopsy. Label each biopsy. Submitting the individual biopsies in tissue cassettes is ideal if you have them. These can be obtained from the diagnostic laboratory. Another method is to place the tissue in a gauze pouch and secure the pouch with string or suture.

CONSIDER performing additional diagnostic procedures: Perform a deep skin scrape to check for

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mites. Skin cytology can be performed by scraping or imprinting. Stain the slide and examine it yourself. Send an air-dried unstained slide to the lab for a clinical pathologist to examine. Would a bacterial or fungal culture be appropriate?

SHIPPING the sample: It is essential to ship this slide in a separate mailing container from the formalin fixed tissue. Formalin vapors from even sealed formalin containers fix the cells on the cytology slide and render the slide useless for cytologic examination. Ship the slide in a box to protect from breakage. DO NOT use cardboard slide mailers in an envelope. The slide will arrive broken. Obtain a fresh tissue biopsy for bacterial or fungal culture if you suspect infectious agents.

DOCUMENT the gross lesion(s): Today, digital cameras, computers, and web access are ubiquitous. Keep a digital camera in your exam room. Print the photos and send with the biopsy submission. Keep a set in the patient's medical record. If your records are completely electronic, keep the digital files in the medical record. Alternatively, email digital images to us: *receiving@vet.k-state.edu*.

Indicate you are submitting biopsies to go with the photos. Identify the patient and owner and we will get the photos into the case file. Use your smart phone to take photos and email us with the same device.

FILL out the submission form completely: You should treat this case as a referral to a histopathologist. Fill out the submission form yourself. The more information your pathologist has, the more helpful they can be. Pertinent clinical information should include lesion distribution and gross characteristics (photos help greatly for this), duration and progression of lesions, presence of pruritus or pain, response or lack of response to therapy, specific drugs used, dose and duration of therapy if known, results of ancillary tests: (CBC, Chemistry panel, Endocrine testing, Cytology, Skin scrape), signs of systemic illness or other medical conditions (pancreatitis, liver disease, neoplasia, etc).

Dermatological conditions are among the most frequent presentations to companion animal practitioners. The skin is the largest organ of the body and is the easiest organ from which to obtain biopsy samples. Gross lesions are visible to the unaided eye without any specialized equipment and are easily documented by photography. Every veterinary practitioner has the ability to obtain high quality skin biopsies and provide a good clinical history so that the practitioner/pathologist team can establish an accurate as possible diagnosis.

Canine Distemper Diagnostics

By Dr. Gordon Andrews

Originally published in the November 2013 issue.

The recent canine distemper outbreak in the animal shelter in Emporia, Kansas has prompted us to briefly review laboratory methods for establishing a definitive diagnosis of distemper virus infection.

The clinical signs of distemper are familiar to most veterinarians and often lead to a tentative clinical diagnosis, but because the clinical signs can mimic other infectious diseases, this review will focus on those laboratory tests that establish a definitive diagnosis. The clinical signs mirror the sequential pathogenesis of viral infection and an understanding of the viral pathogenesis will help determine what samples are most appropriate to select for diagnostic testing.

Exposure to the virus is generally by aerosol contact with epithelium of the upper respiratory tract,

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with multiplication in tissue macrophages and spread by lymphatics to tonsil, retropharyngeal and bronchial lymph nodes. By 4-6 days post infection, virus replication occurs in lymphoid follicles of the spleen, lamina propria of the stomach and intestines, mesenteric lymph nodes, and Kupfer cells of the liver. At this time there is fever and leukopenia. 8-9 days post infection there is hematogenous lymphocyte associated viremia with spread of virus to epithelial and central nervous system tissues with shedding of virus from all body excretions even in dogs with subclinical infection.

Infection of upper respiratory epithelium results in conjunctivitis and rhinitis with serous to mucopurulent occulo-nasal discharge. A conjunctival or nasal swab placed in viral transport medium is an ideal sample at this time to submit for PCR testing for distemper virus. If viral transport medium swabs are unavailable, a swab moistened with sterile saline and placed in a sealed sterile tube is a good substitute. Whole blood in EDTA is an excellent sample for ante mortem PCR testing in both the acute and chronic forms of the disease. Urine is also a good sample for PCR testing.

Infection of lung epithelium results in an interstitial pneumonia, and secondary bacterial infection is common. The resulting cough can mimic infectious tracheobronchitis (kennel cough). A nasal or tracheal swab can be submitted for the canine respiratory PCR panel, which includes canine distemper virus, Mycoplasma, Bordetella bronchiseptica, canine adenovirus type 2, canine herpesvirus type 1, Influenza A, canine parinfluenza-3, and two strains of canine coronavirus.

Infection of gastrointestinal epithelium results in vomiting and diarrhea. Clinical signs of central nervous system infection are dependent on the region of the CNS involved and can include hyperesthesia, cervical rigidity, seizures, cerebellar and vestibular signs, paraparesis or tetraparesis with sensory ataxia, and myoclonus.

Gross necropsy examination often reveals pneumonia, but this is nonspecific. Some dogs develop digital hyperkeratosis (hard pad), but this is not specific for distemper infection. Dogs that have recovered from distemper may have tooth enamel hypoplasia which is considered specific for prior distemper infection. Histopathologic findings can include lymphoid depletion, interstitial pneumonia, necrosis of ameloblastic epithelium, necrosis of epithelium in the gastrointestinal tract, swelling of transitional epithelium in the renal pelvis and urinary bladder, and necrosis and inflammation in the brain. Viral inclusion bodies can be found in epithelial cells of mucous membranes, stomach, intestines, transitional epithelium of urinary pelvis and urinary bladder, neurons and astrocytes. When inclusions are found in association with histologic lesions in a dog with an appropriate clinical history, they can be considered diagnostic. The presence of inclusions alone however must be interpreted with caution, because distemper inclusion-like bodies have been described in the urinary bladder and brain of normal dogs. Distemper inclusions are not always found however and may only be found late in the disease. Immunohistochemical (IHC) staining of formalin-fixed tissues for distemper virus is a sensitive and specific method of demonstrating viral antigen in tissue sections even before histologic lesions are evident.

When submitting necropsy tissues for a diagnostic workup when distemper is suspected is important to include a complete set of formalin-fixed and fresh tissues even if gross lesions are not present and the patient has no clinical signs referable to a particular organ system. As an example, one dog I examined from the Emporia distemper outbreak had gross and microscopic evidence of chronic pneumonia, but PCR testing of fresh lung and IHC testing of formalin-fixed lung were both negative for distemper. There were no other microscopic lesions consistent with distemper infection in any other tissues except the brain, which did have encephalitis, and IHC staining was positive in the brain in spite of the fact that this dog was not reported to be showing any neurologic signs. Fresh necropsy tissues valuable for PCR testing include lymphoid tissues, lung, kidney, and brain.

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Sample Submission for Bovine Abortions

By Dr. Gordon Andrews

Originally published in the January 2015 issue.

We frequently receive phone calls this time of year for advice regarding what samples to submit for bovine abortions, so we are presenting this information as a reminder of the appropriate samples to collect for a thorough diagnostic workup.

Abomasal fluid: Place in a sterile tube and label. This sample is for the bacteriology abortion panel which includes aerobic culture, Brucella and Campylobacter.

Thoracic or pericardial fluid: Place in a sterile tube and label. This sample can be used for fetal serology for BVDV, Leptospirosis, Neospora, Bluetongue, and others. Fetal serology is a valid test to run only during the third trimester of pregnancy when the fetus is becoming immune competent.

Formalin fixed tissues: Place in leak proof formalin container. These tissues are examined microscopically for lesions indicative of an infectious cause of abortion. Brain (sections of cerebrum, cerebellum, midbrain and brainstem), heart, lung, spleen, liver, kidney, adrenal glands, thyroid gland, thymus, and skeletal muscle (3 sections from different muscle groups).

Fresh tissues: Place pooled tissues in a whirlpack bag. Fresh tissues can be used for PCR testing for specific infectious agents (Leptospira, BVDV), bacterial culture, or virus isolation. Lung, liver, kidney, lymph node, spleen, heart, and thymus.

Eye or ocular fluid: Place eye in whirpac bag, or fluid in sterile tube and label. This sample can be used for nitrate analysis if desired.

Placenta: 3-4 sections of fresh cotyledons in a separate whirlpac bag, and 3 – 4 sections of cotyledons in the formalin container. The fixed tissue is examined microscopically for evidence of placentitis. The fresh tissue can be cultured or other procedures such as bacterial culture, PCR, or virus isolation can be performed if there is microscopic evidence of placentitis.

Fresh liver: Place one large sample in a separate whirlpac bag. This sample can be used for trace mineral and vitamin A analysis if nutritional deficiency is suspected as a cause of abortion or weak born calves.

This information as well as many other case specific diagnostic workups is available on the KSVDL website: http://www.ksvdl.org/resources/case-specific-workups/

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The mission of the Kansas State Veterinary Diagnostic Laboratory (KSVDL) is to develop and deliver accurate, innovative, and timely diagnostic and consultative services to the veterinary and animal health community while providing support for teaching, training and research programs.

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