



Newsletter



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From the Director:

A new fee schedule for the Arizona Veterinary Diagnostic Laboratory is being distributed. Many fees have been increased to help compensate for the rising costs of supplies, equipment, and labor. All readers are probably aware that budgets are tight and funding often does not keep pace with costs.

One area with marked increases is molecular diagnostics, which is primarily PCR testing. We have had very low prices on PCR tests while the PCR laboratory was under development. That process is now complete and the laboratory is now conducting routine testing at fees more similar to other veterinary diagnostic laboratories.

We currently offer twenty-five different PCR tests. Also please keep in mind that we will forward specimens to other accredited laboratories if tests that we don't perform are requested.

Robert Glock, Director



Veterinary Diagnostic Laboratory

Diagnostic Services offered at AzVDL:

- ◆ **Pathology:** gross necropsy, histopathology, cytology, or other diagnostic tools used to determine the cause of disease
- ◆ **Microbiology:** the use of microbiological techniques to identify bacteria, viruses, parasites, and other infectious agents, and their relationships to animal diseases
- ◆ **Toxicology:** identification of toxic substances (toxins) and their involvement in animal diseases
- ◆ **Chemistry:** chemical analysis of feed, forage, and body tissue samples into finite compositions
- ◆ **Serology:** analysis of serum to monitor animals' prior exposure to diseases

Arizona Veterinary Diagnostic Laboratory

Access to the AzVDL: Take Tucson Interstate 10 to the Miracle Mile exit #255. Travel approximately 1/4 mile on the south bound frontage road between Miracle Mile and Grant Rd. Turn west onto the farm at the signed entrance.

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Diagnostic Update

The following are selected samples of cases submitted to the AzVDL during the winter months of December, January, and February.

Bovine

Lesions compatible with **bloat (ruminal tympany)** were diagnosed in a seven-month-old Angus heifer that was part of a herd of 35 being fed corn and hay along with a supplement that contained an ionophore (Bovatec®). The owner found the animal dead in the afternoon and the carcass was "very bloated". Necropsy revealed typical lesions of bloat including the following: extensive congestion of the vasculature of the subcutis of the head and neck, peritracheal hemorrhage in the cervical region, pale liver, heart chambers that contained little blood, and a distinct "bloat line" in the esophageal mucosa at the thoracic inlet. PH of the rumen contents was 5.0, which suggests the possibility that a mild ruminal acidosis occurred, resulting in ruminal stasis with subsequent failure of eructation of evolved gas.


A yearling crossbred steer was presented with a history of increasing debilitation over a period of two days. At presentation, the animal had marked nystagmus and severe tetany with response to touching or noise. No lesions were identified at necropsy. This presentation is typical of **tetanus**. There are no practical diagnostic tests for confirmation of tetanus so the diagnosis is based entirely on clinical signs and the lack of other significant changes. Tetanus does not produce

any lesions that are observable grossly or microscopically in the central nervous system. Source of the infection in this animal was not identified. Tetanus can follow relatively small penetrating wounds that are difficult to find. It is also important to note that many of the banding systems used for bovine castration result in increased likelihood of tetanus, particularly in surroundings that have had animal populations in the past. There is some association with previous equine fecal accumulations.

A near-term aborted fetus had multiple necrotic foci in the liver typical of **infectious bovine rhinotracheitis (IBR)**. Fluorescent antibody (FA) test and virus isolation attempts were negative due to advanced autolysis. However, IBR (bovine herpesvirus 1) was still identifiable by PCR. This is a reminder that some cattle populations in this state are not immunized against IBR.


A yearling animal from a feedlot had severe fibrinonecrotic tracheitis. There were also areas of fibrinopurulent bronchopneumonia. **Infectious bovine rhinotracheitis (IBR)** with secondary *Pasteurella multocida* infection was the diagnosis. Fluorescent antibody test for bovine respiratory syncytial virus (BRSV) was positive. However, there were no lesions suggestive of infection with this organism and it was considered to be present but of unknown significance.

Fibrinopurulent bronchopneumonia with numerous *Pasteurella multocida* organisms present was identified in a six-week-old Holstein calf. The anterior ventral one-third of both lungs was con-



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Comments on Diagnostic Update can be directed to Dr. Greg Bradley via e-mail at: azvdl@ag.arizona.edu



Diagnostic Update

solidated and there were areas with a lobular pattern of lesions. No viral pathogens were identified and tests for liver copper, selenium, vitamin A, and vitamin E were within normal limits.

Blackleg was the cause of death of an eighteen-month-old steer from Graham County. The disease was confirmed by direct FA and by isolation of *Clostridium chauvoei*. The infection was septicemic and microscopic lesions of hemorrhagic myositis were observed in several skeletal muscles. The typical gross lesion of black, necrotic myositis was only observed in the heart. It is not unusual for the primary Blackleg lesion to be located in places like the heart, diaphragm, psoas muscle, or even the tongue, where it can be easily overlooked under field conditions.

Equine

Multiple abortions in a herd of twenty-seven Quarter horse mares occurred during a one-week period. Two fetuses were received for necropsy. There was some moderate hepatic and splenic enlargement. One fetus had prominent interlobular edema of the lungs. Histologic lesions included multifocal necrosis and intranuclear inclusion bodies typical of herpesvirus infection in the lungs of both fetuses and in the liver of a single fetus. **Equine herpesvirus 1 (equine rhinopneumonitis virus)** was isolated from the tissues of both fetuses.

Cerebellar abiotrophy was the cause of incoordination and hypermetria in an Arabian filly. The neurologic signs began at about thirty-five to forty days-of-age and remained static until the filly was euthanized at eight months of age. The histologic lesions occurred in the cerebellum and consisted of atrophy of cerebellar folia due to segmental loss of Purkinje and granular cells.

Porcine

Leptospirosis was the cause of abortion in a sow. Grossly the fetuses had scattered subcutaneous hemorrhages and a few pinpoint hemorrhages in the lungs. Spirochetes, morphologically compatible with *Leptospira* sp. were demonstrated in the kidney tubules with silver stains. Serology on pigs from the farm would be necessary to determine the serovar involved.

Small Ruminants

Brucella ovis infection was diagnosed in a flock with a large number of rams with enlarged testicles. The agent was isolated from epididymal abscesses in rams submitted for necropsy. They exhibited chronic, fibrosing epididymitis with abscess formation and adhesions to the adjacent tunics. The rams had been repeatedly vaccinated at the flocks of origin and by the buyer; but had never been tested for brucellosis prior to vaccination and purchase.

Fungal infection due to an unidentified fungal organism was diagnosed in an adult male Cosslet sheep that developed a facial swelling that did not respond to treatment. At necropsy, the facial skin was extensively eroded and crusted with dried serum. The mucosa of the nasal turbinates was discolored light brown. Microscopically, large, septate, dichotomously branching fungal hyphae were evident in central areas of granulomatous inflammation in the skin and nasal turbinate tissue. Fungal cultures, however, were negative and the identity of the offending organism could not be determined.

A chronic active bronchopneumonia with as-

Diagnostic Update

sociated bronchiolitis fibrosa obliterans was diagnosed in a six-month-old Suffolk cross ewe that had been raised on irrigated pasture and fed alfalfa hay. According to the history, the animal was shaking violently, ultimately went down, and developed seizures. A bloody discharge from the mouth was also reported. At necropsy, there was dark red consolidation in the ventral areas of both lungs. Microscopically, there was flooding of alveolar and bronchiolar lumens with mixed leukocytes and fibrin and polypoid intraluminal proliferations of fibroblasts (bronchiolitis fibrosa obliterans) that either partially or completely occluded bronchioles. Plant fragments were occasionally encountered in lung airways. Bronchiolitis fibrosa obliterans (organizing bronchiolitis) is a non-specific response to a variety of severe forms of damage to bronchioles and adjacent alveoli. Chronicity of the lung lesions in this case probably precluded isolation of a causative bacterial pathogen although PCR testing was positive for nucleic acid sequences specific for chlamydia. The occasional plant fragments suggest that aspiration of ingesta may have occurred at some point.

We received an aborted mid-term fetus from a pygmy goat. Infiltrations of inflammatory cells within the lung and placenta plus isolation of *Escherichia coli* from various tissues suggested **abortion due to infection with *E. coli***. This type of infection is considered to be sporadic and is usually not a herd problem.

Avian

A breeder of game birds, including pheasants, quail, and chuckers, was losing twenty-five to thirty birds per day. The birds developed clinical signs that included weakness of the legs and wing flapping. They all eventually died. Gross and microscopic examination did not reveal any significant lesions. The test for **botulinum toxin type c**, using the mouse bioassay, was positive. Textbooks of avian diseases suggest that carcasses of dead birds are the source of botulinum toxin in outbreaks of this type, either directly due to con-

sumption of the carcass or indirectly due to consumption of insects/larvae feeding on the carcass. *Clostridium botulinum* spores germinate in the decaying flesh of the dead birds and produce toxin. The birds or insects thus consume the toxin in a pre-formed state. The owner insisted that no dead birds were allowed to collect in the pens making this scenario unlikely. This suggests that the described outbreak represents the "toxico-infectious" form of botulism. In this form of botulism, spores present in the environment (soil) are ingested with subsequent germination in the gastrointestinal tract followed by production and absorption of the toxin. A similar scenario is thought to be the cause of "shaker foals", due to *Clostridium botulinum* type B.

One hundred and fifty sparrows were found dead during a six-week period in a Phoenix residential neighborhood. It was noted that some of the birds were blind in one or both eyes prior to death. Gross lesions included focal pseudomembranous ulcers in the wall of the esophagus and splenomegaly. Group B *Salmonella* sp. was isolated in large numbers from the tissues of the birds. **Salmonellosis** is a sporadic cause of large-scale mortality in wild birds.

Ventricular impaction by a three-inch section of a toy rope was identified as the cause of death of an Umbrella cockatoo. Although the history indicated "acute death without showing any prior signs of distress", the rope appeared to have caused emaciation of the bird by completely filling the ventriculus and interrupting the passage of ingesta. Tests for heavy metals were negative.

Hepatitis due to an unidentified viral infection was diagnosed in a pigeon identified as a Roller. Ten of fifty died after being sick for a week over a period of two months). At necropsy there was enlargement of the spleen. Microscopically, there was hepatocellular degeneration and most hepatocytes contained eosinophilic intranuclear viral-type inclusions. Prominent lymphoid depletion was

Diagnostic Update

evident in the spleen. Nucleic acid sequences specific for chlamydia were also identified by PCR testing of liver, lung, and spleen.

Candidiasis of the crop was diagnosed in a seven-day-old cockatiel. Focal mucosal necrosis and ulceration accompanied by heavy colonization of yeast-type organisms with morphology compatible with that of *Candida* sp. was found in sections of the crop. No other pathogens were found.

Feline

Feline herpesvirus pneumonia was the cause of death in a two-week-old Maine Coon cat. It was the second of a litter to die following onset of respiratory signs. Gross lesions consisted of areas of pink/tan consolidation of the lung lobes.

A **mild hypertrophic cardiomyopathy** was the most significant finding in the necropsy of a seven-year-old neutered male Persian feline, which died of cardiac arrest after anesthesia for dental cleaning. Nodular thyroid hyperplasia and a mild glomerulonephritis were also found on microscopic examination of the tissues. The cardiac lesions were the most likely reason for the cardiac arrest.

The cadaver of a seven-month-old Siamese kitten was received for necropsy with a history of an upper respiratory infection, which progressed to neurologic disease. Necropsy findings included two unrelated infectious processes: a granulomatous meningoencephalitis of the cerebellum and brain stem with changes characteristic of non-effusive **Feline Infectious Peritonitis**, and a chronic pneumonitis caused by *Chlamydophila felis* (as demonstrated by PCR of lung sections).

Non-effusive **Feline Infectious Peritonitis** was also diagnosed in a one-year-old feline, which died with perirenal edema and hemorrhage caused by severe subcapsular vasculitis and perivasculitis with thrombosis, infarction, and necrosis.

A **nasal adenocarcinoma of the frontal sinuses with extension into the frontal lobes of the cerebral cortex** was diagnosed in an adult domestic feline that was submitted from a cat shelter. At necropsy, there was external evidence of heavy flea infestation and the frontal sinuses and frontal lobes of the cerebrum contained reddish-gray, tumorous tissue. In addition to this finding, the animal had focal septal myocardial necrosis, a multifocal necrotizing pancreatitis, and mild hepatic lipidosis.

Canine

Chronic adrenalitis with fibrosis and atrophy of the cortex consistent with **Addison's disease** was the diagnosis in a ten-year-old Schnauzer. The dog had normal blood work prior to undergoing a dental prophylaxis. It was slow waking from anesthesia. Initially it did well at home but then became progressively depressed during the next two days and died.

A septicemia caused by **group G Streptococcus sp.** was the cause of death in a four-month-old Chinese Crested dog. The puppy had a history of ecchymotic hemorrhages in the skin and a negative *Ehrlichia canis* titer. At necropsy there were ecchymoses seen in the skin, skeletal muscle, pleura, peritoneum, kidney cortex, mucosa of the stomach, and lungs. The tonsils were swollen, dark red, and had extensive pale foci (tonsillitis). On histologic examination, large colonies of streptococcal-type bacteria were visible in the tonsils with focally extensive areas of necrosis.

Pulmonic stenosis was found in a young, emaciated female Chihuahua. There was a marked hypertrophy of the right ventricle and a dilated right atrium. There was marked pulmonic stenosis with fibrotic valves and a post-stenotic dilatation of the pulmonary artery. Pulmonic stenosis is a congenital cardiac anomaly suspected to be hereditary in Chihuahuas.

Diagnostic Update

Canine herpesvirus infection was diagnosed in a litter of puppies that had been euthanized after an illness of unspecified nature. At necropsy, there were multifocal, tiny, white lesions scattered throughout the parenchyma of the livers. Subcapsular hemorrhages were evident in both kidneys of two of the puppies. In sections of liver, there were multiple foci of hepatocellular necrosis and in two of the puppies there was multifocal necrosis of renal tubular epithelium in the cortex. All puppies had severe diffuse interstitial pneumonia. Mild lymphocytolysis was evident in some lymphoid follicles of spleen. PCR testing of liver tissue from all of the puppies was positive for nucleic acid sequences specific for canine herpesvirus infection.

Canine herpesvirus infection was diagnosed in a six to eight-week-old Labrador mix puppy, which died after a short clinical course of diarrhea. Intracellular viral inclusion bodies and lesions of focal necrosis, characteristic of the virus, were observed in the liver, kidneys, and lungs. In addition, there was an intestinal cryptosporidiosis. Typically, the generalized, lethal infection of puppies by canine herpesvirus occurs in younger puppies. The virus replicates best at 33° C, and therefore it is more likely to affect puppies under four weeks of age before hypothalamic thermoregulation becomes effective. In this case, excessive exposure to low ambient temperature may have resulted in hypothermia, perhaps aggravated by the cryptosporidium-associated diarrhea.

Severe diffuse pyogranulomatous peritonitis due to *Mesocestoides* infection was diagnosed in tissues from a seven-year-old neutered male Bassett hound. The lesions, described by the referring veterinarian, consisted of "cysts in the abdomen". The cyst-like structures were identified as tetrathyridia of *Mesocestoides* by a parasitologist at another diagnostic laboratory. Microscopically, there was prominent proliferation of serosal mesothelium that was accompanied by severe

pyogranulomatous inflammation. It is reported that tetrathyridia, the larval form of the tapeworm *Mesocestoides*, may proliferate extensively in the abdominal cavity of carnivores where they cause a characteristic pyogranulomatous peritonitis. The life cycle of *Mesocestoides* is described as requiring two intermediate hosts. The first is a coprophagous insect in which a cysticercoid is produced. When the infected insect is eaten by a second intermediate host, (dog, cat, etc.) a tetrathyridium is formed. This is a slender worm-like structure one to two centimeters in length. When the final host eats the tetrathyridium, it becomes an adult in sixteen to twenty days. Tetrathyridia have been reported to become encapsulated in the retroperitoneal and subcutaneous tissues or to be free in the abdominal and thoracic cavities. There are reports of tetrathyridia occurring in the scrotum, bladder, liver, intestines, and rarely in the lungs of mammals, reptiles, birds, and amphibians.

Lymphoplasmacytic enteritis (inflammatory bowel disease) was diagnosed in a five-year-old female Rottweiler. It was euthanized after extensive treatment for "protein losing enteropathy". Prior to euthanasia the dog had a total protein of 2.0 g/dl and a hematocrit of 20%. At necropsy, the abdominal cavity was full of ascitic fluid that contained flecks of fibrin-like sediment. Loops of small intestine were pale, opaque, dilated, and had thickened, soft walls. Microscopically, diffuse infiltrates of plasma cells were present in the lamina propria of mucosa with resultant distention of villous tips with lymphoid cells. Villous effacement was evident in some cases. Some mucosal crypts contained eosinophilic material and a few necrotic sloughed cells. Fibrosis of the cortices of both adrenal glands was also evident.

Canine hemorrhagic gastroenteritis (canine gastrointestinal hemorrhage syndrome) due to *Clostridium perfringens* type A infection was d-

Diagnostic Update

agnosed in a three to four year-old Husky-Malamute mix. The history provided with the case was vague but poisoning was suspected. At necropsy, there was dark-red fecal soiling on the under side of the tail. The entire small intestinal lumen contained dark red fluid. Stomach contents consisted of turbid, semi fluid material. Microscopically, post-mortem decomposition obscured considerable detail but gram stained smears of small intestinal contents revealed large numbers of gram-positive rods along with other flora. Anaerobic cultures yielded *C. perfringens* type A. Screening for toxicants and parvovirus was negative. Canine hemorrhagic gastroenteritis is reported to occur sporadically and is usually peracute, with affected dogs often found dead, lying in a pool of bloody excreta. Hemorrhagic diarrhea prior to death is also sometimes reported.

A necrotizing pancreatitis was diagnosed in a six-year-old male Dachshund that died following an illness of about two weeks duration. The dog was seen initially for a "painful back" and responded somewhat to treatment. It was presented approximately a month later for vomiting and diarrhea. In-clinic Parvo testing was negative. The white blood cell count was 16,000/ μ l. The dog worsened over night and began vomiting digested blood. By the second day the white blood cell count had risen to 24,000/ μ l. Serum glucose was 410 mg/dl. At necropsy, there were multiple foci of opaque, white discolorations of peripancreatic fat. Microscopically, there were foci of necrosis in the pancreas, interlobular edema, vacuolation of islet cells, and prominent infiltrates of mixed leukocytes in peripancreatic fat. Lipidosis was evident in the liver.

Canine parvovirus infection was diagnosed in a nine-week-old male Pointer mix that was fine in the AM, but later was having difficulty breathing and was urinating blood. Necropsy revealed extensive fecal soiling of the perineal hair. Serosal vessels of the small intestine were hyperemic. Gut contents were scant. Microscopically, the lesions were typical of canine parvovirus infection and PCR testing of the spleen and small intestine was positive for nucleic acids sequences specific for the virus.

We received a mature Dachshund with a clinical history suggesting CNS disease. The dog also had a history of intervertebral disc disease. Disc material was found in the spinal canal at the level of L₃ and L₄. No other significant lesions were observed grossly but there was extensive diffuse granulomatous meningoencephalitis in the brain and some segments of the spinal cord. The diagnosis was **granulomatous meningoencephalitis**. Etiology of this condition is unknown.

Diagnostic Update

A five-week-old Labrador puppy was presented for necropsy with a history of sudden death. The diagnosis was **asphyxiation** resulting from aspiration of dog food into the larynx and trachea.

An aneurysm of the pulmonary artery was the cause of debilitation and some weakness in a ten-week-old puppy that eventually died. Necropsy findings included 15ml of clear yellow ascitic fluid, and an aneurysm just distal to the semilunar valve in the pulmonary artery. There was also cardiac dilatation. The cause of this dissecting aneurysm, which was approximately 1cm in length, was not determined.

Exotics

Iron Storage Disease was diagnosed in a yellow-bellied Greenbul (bird) from a zoological park. No clinical signs were observed prior to death. There was vacuolation and necrosis of individual hepatocytes, with strong staining of the hepatocytes by Prussian blue, which is indicative of excessive iron. The iron content of the liver was 2847 ppm (reference value is approximately 300 ppm).

Hemosiderosis of liver and gut mucosa was diagnosed in a four-and-one-half-year-old female Calliope hummingbird from a zoo. Special stainings revealed positive pigment in the cytoplasm of most hepatocytes and in gut mucosal cells. Liver tissue was reported to contain an excessive amount of iron (1,322 ppm; reference value is approximately 300 ppm).

Severe, diffuse, hepatocellular lipidosis was diagnosed in a four-year-old ferret. The animal reportedly developed "anorexia, vomiting, diarrhea, and lethargy". At necropsy, the liver was diffusely discolored pale yellow.

We received a Fruit dove with a history of being found dead. The bird was somewhat thin and the gastrointestinal tract was empty. There were his-

tologic lesions in the liver including foci of hepatocellular degeneration and inflammatory infiltrate with some brown-pigmented macrophages present. Liver iron level was 3,204 ppm. This appears to be a case of **hemochromatosis**. The cause of iron accumulation in this bird is not determined.

Wildlife

Hemorrhagic disease due to concurrent infection by BTV and EHD was diagnosed in a male mule deer. Patches of alopecia were evident along the lateral aspects of the trunk of the animal. A few abrasions were evident on the skin of the hind legs. No other external lesions were noted. Internally, the carcass appeared to be underweight with minimal depots of subcutaneous fat. Rumen ingesta consisted of grass and shrub-type material containing a few leaves. The trachea contained some froth. There was patchy, dark-red discoloration of the lungs. A hemorrhage was present at the base of the aorta. A few small hemorrhages were evident on the papillary muscles of the left ventricle of the heart. Brownish, fetid, semi liquid material was present in the lumens of small and large intestine. Fecal pellets were absent in the lower colon. Locally extensive hemorrhage was evident in the semimembranosus/tendinosis musculature of the left hind leg. There was excess cerebrospinal fluid. Microscopic lesions were typical. PCR testing was positive for both bluetongue (BTV) and epizootic hemorrhagic disease (EHD) viral RNA. Simultaneous infections by BTV and EHD in animals have been reported in wildlife literature. Another mule deer buck from the same area was received several weeks after this case. That buck also had lesions typical of hemorrhagic disease. PCR testing in that case was positive only for EHD, not BTV.

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