



Newsletter

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Quarterly Publication of the **Arizona Veterinary Diagnostic Laboratory** and Cooperative Extension

AzVDL Receives Accreditation

The American Association of Veterinary Laboratory Diagnosticians is a professional association of laboratory workers engaged in the field of disease diagnosis in animals. It disseminates information on the diagnosis of animal diseases, it coordinates diagnostic activities by laboratories, and it fosters the improvement and standardization of diagnostic techniques. It also establishes guidelines for the improvement of diagnostic laboratories relative to personnel qualifications and facilities. The Board of Accreditation of the AAVLD at its last meeting in October of this year, accredited the Arizona Veterinary Diagnostic Laboratory as a full service veterinary diagnostic laboratory for all species. This accreditation is a national endorsement of our capabilities of providing a full range of diagnostic services to the State of Arizona. The Arizona Veterinary Diagnostic Laboratory is now one of 36 such accredited labs in the US and Canada. We at AzVDL consider it an honor as well as a challenge to continue providing our best efforts on behalf of animal health and welfare throughout the state.

Carlos Reggiardo, Director

Profile of the AzVDL Histology Section

The histology section of the laboratory produces glass slides of stained tissue sections from submitted specimens. These include biopsies, field necropsy tissues and tissues collected from necropsies performed at the AzVDL. In addition, high quality histology sections are provided to members of the University of Arizona research community with research programs utilizing animal tissues. On average, the laboratory produces 400 slides per week.

The laboratory is staffed by two registered technicians having a total of 57 years of experience in histology. Esther Kerr has been with the University of Arizona for 8 years. Jan Mac Millan has just recently been employed at the AzVDL but, excepting a brief stint as a histotechnologist at a local human hospital, was employed by other departments of the UA for 17 years.

In addition to routine hematoxylin and eosin (H&E) stained sections, the laboratory has the capability to

produce numerous special histochemically stained tissue sections for use in differentiating infectious agents, pigments, metabolic products, etc.

Processing for electron microscopy and frozen sectioning are available by arrangement. Please contact the laboratory before submitting.

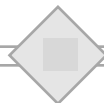
In general, adequately fixed tissues arriving at the AzVDL before 4:30 PM are trimmed by a diagnostician, with the assistance of a histotechnologist, and processed overnight. Slides are usually cut, stained and ready for examination by the diagnosticians by 1:00 PM the following day. Inadequately fixed specimens and bones or other hard specimens requiring decalcification will require one or more additional days for processing. Tissues collected from necropsies at the AzVDL are routinely held overnight in formalin before trimming and processing to ensure adequate fixation and thus high quality, readable slides. Practitioners can obtain replicate sections of their submitted specimens upon request. Just indicate on the submission form that you want an additional H&E section and/or specially stained section. Your account will be billed \$10/slide plus postage. Researchers or other laboratory clients anticipating the need for high volume histology services should contact either Esther Kerr or Jan Mac Millan at 621-2356 regarding special pricing.

Submission Tips

Inadequate fixation is a common problem. In general, a 10:1 volume of formalin to tissue is required to insure adequate penetration of the tissue and fixation of deeper areas. Larger volumes (30:1) are required for tissues with a high water content such as brain. Additional formalin is also necessary to adequately fix extremely bloody specimens such as spleens or hemangiosarcomas from any site. Ideally, tissue should be no thicker than 5 mm. Large

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specimens should be incised serially at 5 mm intervals to allow proper penetration of fixative. Brains and spinal cords are exceptions to this rule. Sectioning of these tissues before fixation will introduce unwanted artifacts.

Packaging of formalinized tissue requires special care.

Tightly sealed containers, either glass or plastic, are preferred to whirl-packs or baggies which almost always leak. Urine specimen containers are ideal and are usually available from medical supply vendors. When multiple lesions (i.e. tumors) are removed from a patient, the lesions should be placed in separate containers and the collection site marked on the label. The submission form should be placed in a **separate** plastic bag to prevent obliteration by leaking formalin. For specimens which are not urgent, the tissue may be fixed overnight in an adequate volume of formalin, then removed, wrapped in a formalin soaked gauze 4X4, and placed in a container for shipment. This will minimize the chances of formalin leakage.

Care should be taken to match the size of the specimen to the size of the opening of the container. A large tumor which, when freshly excised, is easily slid through the narrow opening of jar will harden with fixation and be impossible to extract from the container when received at the lab (The so-called breast in a ketchup bottle phenomenon). We keep a hammer in the laboratory to break glass containers containing oversize specimens but flying glass presents a hazard and breaking containers is best avoided.

Diagnostic Update

The following are selected samples of cases submitted to the AzVDL during the months of September, October and November.

Canine



Lymphocytic thyroiditis was diagnosed at necropsy of a one year-old doberman which died of respiratory failure during anesthesia.

The inflammatory process destroyed most of the follicular architecture of both glands. Presumably, deficiency of thyroid hormone production rendered the dog more sensitive to anesthesia. Hashimoto's thyroiditis (which has an inherited basis) is a similar disease in humans. Autoantibodies against thyroglobulin, a microsomal antigen and a second colloid antigen are the immunologic basis of the disease. Autoantibodies against thyroglobulin have been found in a high percentage of dogs with lymphocytic

thyroiditis. A similar, polygenic pattern of inheritance is suspected.

Idiopathic adrenocortical atrophy and lymphocytic thyroiditis were diagnosed in a two year old standard poodle which died of cardiovascular collapse after several days of gastroenteritis and a history of weight loss. The hypoadrenocorticism was believed to be the actual cause of death. As is the case for lymphocytic thyroiditis, the pathogenesis of idiopathic adrenocortical atrophy is suspected to be immune mediated. The coexistence of both conditions in a young adult dog is intriguing and lends credence to the possibility of an immunological basis in the production of the diseases.

Complications related to **heat stroke** led to euthanasia of a fourteen-month-old golden retriever. The dog collapsed acutely while running with the owner. The owners attempted to cool the dog by wetting it with a garden hose. At presentation, the rectal temperature was 104.5° F. The alkaline phosphatase, AST, BUN, creatinine, total protein, globulin and WBC were all elevated. Platelets and blood glucose were decreased. Bruising was noted on the skin. The dog was vomiting and the vomitus contained blood. Treatment with intravenous fluid, metronidazole, dexamethasone, oxytetracycline and ampicillin was given. The following day, the lab values were worsening. The BUN and creatinine were markedly elevated and the stool was bloody. Seizures developed. Euthanasia was elected by the owner. Necropsy examination revealed acute renal tubular necrosis, myocardial necrosis, acute neuronal necrosis and cerebral edema, vacuolar degeneration of periportal hepatocytes and petechial and ecchymotic hemorrhages of the subcutis, diaphragm and serosal of abdominal viscera. Multiorgan failure and DIC are common complications of heat stroke. An excellent retrospective study of heat stroke in dogs including clinical presentation, laboratory data and prognostic indicators recently appeared in JAVMA, Vol 209, No.11, pp. 1894-1899.

Intestinal incarceration was the cause of death in a female poodle. Death occurred after brief clinical illness characterized initially by vomiting followed by progressive deterioration and coma. Lab work revealed a blood glucose of 39.8 mg/dl. Prior history included a cesarean section performed two months prior to presentation. At necropsy, a 43 cm long section of jejunum and ileum was looped over a 1 cm long adhesion extending between the uterus and the serosa of small intestine (mid-jejunum). The bowel was black, dilated and filled with watery red fluid.

Two cases of **canine distemper** were diagnosed in puppies from two separate households. Clinical signs included paresis and purulent nasal discharge.

Septicemia due to *Klebsiella* sp. Infection was the cause of death in a three-day-old puppy. This was one of three puppies which “faded” shortly after birth.

Severe intrathoracic **hemorrhage** was found during the necropsy of a 3 year-old female Labrador mix. Locally extensive hemorrhages of the pericardium, perirenal fat, and skin and subcutis of the right forearm and left inguinal area were also found. There was a history of use of anticoagulant-type rodent bait by the owner on the premises where the dog was kept. The lesions could be considered compatible with ingestion of anticoagulant substances of this type but the owner refused additional tissue analysis to confirm this diagnosis. The case does, however, confirm the necessity of taking the time to develop an adequate history, particularly in cases of unexpected death of otherwise apparently healthy animals.

Severe diffuse chronic glomerulonephritis resulting in **secondary hyperparathyroidism with resultant fibrous osteodystrophy** was diagnosed in an 8 year-old keeshond. Both the maxilla and mandible were flexible and the calvarium was soft and fibrous. The parathyroid glands were enlarged and prominent. Microscopic examination revealed hyperplasia of the parathyroid glands and osteoclastic activity was evident around remnant spicules of bone in a section of mandible.

A ruptured splenic **hemangiosarcoma** was the cause of death in an eight-year-old, spayed female, german shepherd dog which presented for acute onset of depression and lethargy. Clinical findings before death included pale mucous membranes, abdominal distention and thrombocytopenia.

Pheochromocytoma of the right adrenal gland was the cause of death in a seven-year-old female maltese. The initial presentation was for polyuria and polydipsia. The dog died while hospitalized for treatment. In addition to the adrenal tumor, left ventricular hypertrophy was noted at necropsy. Most pheochromocytomas are productive. Excess catecholamine production is responsible for the cardiac hypertrophy and can also induce cardiac arrhythmia. The latter was the presumed cause of death.

Actinomyces viscosus was cultured from the chest cavity of a three-year-old, female, hound with a severe, bilateral, proliferative pleuritis and pyothorax.

Coccidioides immitis was the cause of severe pericarditis, ascites and pleural effusion in a three-year-old irish wolfhound which exhibited acute onset of respiratory distress.

A six-year-old, castrated male, german shepherd dog was

presented for paraparesis, incontinence, weight loss and anorexia. A severe **granulomatous peritonitis** due to *Coccidioides immitis* was found at necropsy.

Feline



Three separate cases of **pyothorax** in cats were recorded in this quarter. One was caused by *Bacteroides* sp., one by *Nocardia asteroides* and one by *Pasteurella multocida*. Two of the cats had been tested negative for both feline immunodeficiency and feline leukemia viruses. The third cat was not tested.

A **rhabdomyosarcoma** developed at the site of a prior vaccination in a fourteen-year-old, castrated male domestic shorthair cat.

Ethylene glycol poisoning was the cause of neurologic signs in a one-year-old, female domestic shorthair cat which subsequently died after a three day history of illness. The signs included, seizures, head twitching, circling and deficits in the left foreleg. The BUN, creatinine and serum phosphorus levels were all elevated.

Equine



Oleander poisoning was diagnosed in a seven-year-old, arabian mare. The horse exhibited clinical signs of colic. The pulse was bounding and 110 beats per minute. Ileus and cyanotic mucous membranes were present. The stomach and cecal contents tested positive for oleandrin.

Vegetative endocarditis followed by myocardial infarction was the cause of sudden death in a four-year-old miniature horse mare. She had been treated for a snotty nose two weeks prior to death. *Streptococcus equi subsp. zooepidemicus* was cultured from the endocardial lesions, spleen, small intestine, lung and kidney.

Aeromonas hydrophila was cultured from a sicilian donkey fetus which aborted at three months gestation. The jenny was in deep shock but responded to antibiotic, corticosteroid and fluid therapy.

Abortion due to a **mycotic placentitis** was diagnosed in an arabian mare. Although no significant gross or microscopic lesions were observed in the aborted fetus, the placenta was thickened and covered by a fibrinopurulent exudate. Microscopically, there was a severe necrotizing placentitis with numerous fungal elements. The fungal agent was a dermatiaceous mold with septate hyphae, an opportunistic agent which most likely gained entry to the

uterus through a relaxed (open) cervix, a relatively common occurrence during equine pregnancy.

Bovine



Phosphorus deficiency was diagnosed in two cows submitted for necropsy examination. Both cows exhibited ill-thrift and forelimb lameness.

They were grazing pastures rehabilitated after a mining operation. The herd had low serum phosphorus values (1.5 to 1.8 $\mu\text{m}/\text{dl}$). Adequate serum phosphorus ranges from 4.5 to 6.0 $\mu\text{g}/\text{dl}$. At necropsy, ribs were easily broken. Histologically there was osteopenia, osteomalacia and microfracture. Analysis of pasture grasses revealed low phosphorus levels (500 to 1500ppm). Forage phosphorus levels should be at least 3000 ppm. Supplementation of the cows with alfalfa hay resulted in a rapid clinical improvement.

Several cows recently placed in a new pasture exhibited clinical signs of tremors, collapse, convulsions and rapid death. The owner reported that similar death losses had occurred in the same pasture in prior years. As many as 35 animals had died during one incident. Necropsy examination revealed no specific gross or microscopic lesions. Pasture plants were collected for identification. Two species of milkweed, *Aesclepias subverticillata* and *A. asperula* were identified. **Milkweed poisoning** was confirmed after the identification of milkweed plant parts in rumen contents by the Range Laboratory.

Two cows and four calves were found dead on desert pasture in Maricopa county. All cattle were in good body condition. There was abundant grass available but it was dry. It was noted that the cattle were largely ignoring the grass and grazing weeds and bushes which were green. Microscopic examination of liver from one field necropsy revealed severe, diffuse, centrilobular and midzonal hepatocellular necrosis. A field investigation revealed cocklebur along a wash in the pasture. Analysis of rumen contents at the Range Laboratory to confirm **cocklebur poisoning** is pending at this time.

Porcine



Water deprivation was diagnosed in a 3 month old pig that was submitted for necropsy. The owner suspected this as the animal was among a group of recently-purchased pigs put in a pen with a automatic-type watering system with which they were unfamiliar. The animals were not checked for a period of 2 days and when the owner returned he found the pigs “shaking” and “blind”. He also reported that the animals would “go into corners”. Brain sodium levels were markedly elevated (3167 ppm in this animal), <1800 ppm is normal. A characteristic eosinophilic perivasculitis was present in the brain.

Exotic and Wildlife



Multiple cases of **canine distemper virus** infection in javelinas have been seen. The animals have come from the northern, central and southern portions of Arizona. Clinical signs usually include ataxia and abnormal behavior.

Epizootic Hemorrhagic Disease was diagnosed in two mule deer from the Kingman area. Both animals were found dead near a residential area. One had been observed with seizures a few days prior to death. Pertinent necropsy findings included multifocal edema, hemorrhage, ulcers on the lingual mucosa and non-suppurative encephalitis. EHD virus was isolated from the spleens.

Metastatic mineralization of multiple tissue was diagnosed in an adult female panther chameleon. This condition is most often due to oversupplementation with vitamin D to prevent metabolic bone disease in reptiles.

A **malignant plasma cell tumor** of the small intestine, pancreas, kidney and hepatic lymph node was diagnosed in a six-year-old female ferret presented for lethargy and vomiting.

A mule deer doe from the Grand Canyon National Park was euthanized for emaciation and hind limb paresis. At necropsy, the left kidney was twice normal size and the renal pelvis was filled with purulent yellow exudate. The uterus was dilated, thickened and filled with similar exudate. The wall of the urinary bladder was slightly thickened. Microscopically, there was a severe, purulent cystitis and pyelonephritis. *Corynebacterium renale* was isolated.

Selenium deficiency and **Chlamydiosis** were diagnosed in a two day old chacoan peccary which died suddenly. Hepatic necrosis, myocardial necrosis and gastric ulceration were the main lesions observed.

Avian



Cryptosporidiosis was the cause of high mortality in two flocks of three-week-old, ostrich chicks. Clinical signs included anorexia, lethargy and diarrhea.

A six-month-old emu presented for necropsy had a history of gradual onset of lethargy, anorexia and ataxia. A **lysosomal storage disease** was diagnosed at necropsy based on characteristic neuronal vacuolation in the brain and aggregates of vacuolated macrophages in the liver. This disease has only recently been reported in the literature. Biochemical studies are incomplete, but a

gangliosidosis is suspected based on ultrastructural and histochemical characteristics of the storage material. An inherited basis for the disease is probable.

A **fibrosarcoma** of the nasal cavity was the cause of nasal discharge in a six-month-old female parakeet.

Sudden death in a female scarlet ibis from a zoo collection was due to heart failure resulting from **vegetative valvular endocarditis**. The lungs exhibited lesions of a prior respiratory infection and was the probably source of the valvular infection. Gram-positive coccoid bacteria consistent with a *Staphylococcus sp.* were visualized in the valve lesion.

Parathyroid gland hypertrophy was found at necropsy of a 15 year-old female african grey parrot which exhibited acute onset of ataxia and seizures. Parathyroid hyperplasia in psittacines is suggestive of inadequate calcium in the diet (nutritional secondary hyperparathyroidism). A diet consisting almost entirely of seeds is a common history. Hypocalcemia leads to seizures.

Avian tuberculosis was diagnosed in two birds submitted to the AzVDL for necropsy. The owner of the first bird, a pacific parrotlet, reported that the bird seemed “weak” the day before its death. Grossly apparent lesions included an enlarged spleen and fibrinous adhesions between the liver and adjacent viscera. Microscopic examination revealed nodular accumulations of large, swollen macrophages

which contained acid-fast bacteria in liver and lamina propria mucosae of, which are the most commonly found lesions in birds. A second case of avian tuberculosis was diagnosed in a green singer finch, which had experienced pronounced weight loss prior to death. Microscopically the mycobacteria infection was confined to the atrium of the heart, the testicles, and to a single small focal lesion in the lung.

Salmonellosis was diagnosed in 5 of 6 avian (budgies) cadavers submitted for necropsy to the AzVDL. The sixth bird had lesion compatible with salmonellosis but the organism was not isolated from its tissues. All birds were from the same aviary. Submission of multiple cadavers for necropsy of birds dying during an outbreak of unknown disease is preferable to a single bird submission. Packaging specimens in leak-proof plastic bags and packing them in ice for initial cooling prior to shipment is the preferred method of handling these cases. Changing the wet ice for leak-proof “ice packs” should be done for actual shipment in an insulated container. The zoonotic hazard from contagious diseases such as salmonellosis and chlamydiosis should always be kept in mind and leak-proof packaging and sanitation should be emphasized to owners and technicians handling these specimens.

Greg Bradley, T. H. Noon, Carlos Reggiardo

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

Zoonosis Update

The Arizona Department of Health Services has recently reported a severe case of intestinal salmonellosis in a 6 week old Phoenix infant linked to the family’s pet iguana. *Salmonella stanleyville*, a rare serotype of salmonella was isolated from both the baby and the iguana. Reptile associated salmonellosis is a growing public health concern. CDC estimates that between 3 and 5 % of the cases of human salmonellosis in the US may involve pet reptiles. To avoid the risk of salmonella infection from pet reptiles, the ADHS has recently released the following guidelines:

1. People at increased risk of infection or serious complications should avoid contact with; reptiles. This includes children younger than 5, and immune-compromised people such as HIV infected patients, persons receiving immunosuppressive drug therapy, and the elderly.
2. Thoroughly wash hands with soap and water immediately after handling reptiles or cleaning their cages.
3. Reptiles should not be allowed to crawl around on the floor where children play. Keep pet reptiles out of food preparation areas, such as kitchens, to prevent contamination of surfaces. Do not bathe reptiles or wash cages, aquariums, water bowls, etc. in kitchen sinks. Disinfect reptile exposed surfaces.
4. Antibiotics should not be given to pet reptiles to eliminate salmonella or other bacteria commonly found in these pets, as this can result in the proliferation of antibiotic resistant strains of bacteria.

Arizona Veterinary Diagnostic Laboratory

By vehicle the only access to the AzVDL is via Prince Road (I-10 Tucson exit 254), south on La Cholla to the West Campus Agricultural Center Farm. Follow the signs and the dirt road to the facility.

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An electronic version of the AzVDL Newsletter can be accessed at: <http://microvet.arizona.edu>

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