



Newsletter

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From the Director:

The AzVDL has an extremely varied case-load. My prior experience in several state diagnostic laboratories has not exposed me to such variety. We welcome this mix of exotic birds and animals but they present some interesting challenges. Reliable information is scarce so we are very dependent on good histories and tips from practitioners who often know a great deal about these species and who often have a little different perspective. This laboratory must accept submissions from owners. We try very hard to get a practitioner involved because it is neither appropriate nor prudent for us to provide clinical advice. Practitioner input on urgency also helps us. In summary, we welcome exotic animal submissions and we will do our best with them. We encourage ongoing input and information from the interested veterinary community.

Robert D. Glock

New mass spec in toxicology

The AzVDL now has enhanced capability to identify certain types of organic toxicants. This capability has been made possible with the addition of a computerized mass spectrometer (MS) to one of our existing gas chromatographs (GC). The resulting GC/MS with a computerized library of spectra for many thousands of organic compounds provides us with an exceedingly powerful diagnostic tool.

The technique is especially useful for screening and identifying most drugs, pesticides, certain rodenticides, and industrial chemicals in tissues and body fluids. However, like all analytical techniques, it has some limitations. For example, this method is not applicable for heavy metals, very high molecular weight organic molecules (e.g. monensin), some rodenticides, and peptide-alkaloids (e.g. amanitin from toxic mushrooms) and glycosides such as oleandrin (from oleander). Other analytical techniques are available to us for the detection of these exceptions.

This enhanced technique is of value to you, our clients, because it gives us the capability to identify affordably a large number of toxicants that would not have been readily identifiable using previous methodology. It must be remembered, however, that the GC/MS technique only supports traditional diagnostic methods with the usual rule-in/rule-out procedures. A good history, and in the case of animal death, thorough necropsy procedures to rule out other types of disease should precede a decision to screen for organic toxicants using GC/MS methodology.

Interpretation of GC/MS data is expedited considerably if the submitting veterinarian provides a good history, especially with regard to known or suspected drug usage or chemical exposure. Please feel free to contact us for more information on how this technique might be of value to you.

by T. H. Noon and Dana Perry Betzer

Diagnostic Update

The following are selected samples of cases submitted to the AzVDL during the summer months of June, July and August.



Bovine

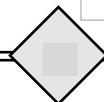
Anaplasmosis was diagnosed in a group of feedlot steers exhibiting anemia and death.

Typical anaplasma bodies were seen on blood smears in erythrocytes. Infection was confirmed in nine animals by the complement fixation test.

Twelve cows died in a herd of 350 that were grazing a pasture having ample feed, but which also had a dump area containing construction wastes. Some of the remain-

In this issue:

From the new director	page 1
New instrumentation - Mass spectrometer ...	page 1
Diagnostic case update - June to August.	page 1
Botulism	page 5



ing sick cattle were partially blind. The submitting veterinarian suspected **lead poisoning**, and this was confirmed in one of the cows. The cow had elevated blood levels of lead (0.65 ppm, toxic range > 0.35 ppm) Another cow had a **pharyngeal impaction** caused by a hard, rubber-like disk which resembled the backing used for sanding disks used in the construction trades. The cow was also thin and emaciated. Examination of the rumen contents from both cows revealed assorted bits of rubber-like material, multicolored, linoleum-like material, and yellowish-white, irregular bits of plastic-like material. The cow with the pharyngeal impaction also had elevated levels of zinc in its liver (248 ppm, normal range 25-100 ppm). These cases illustrate the hazards associated with construction dumps. Cattle are curious and seem to have a propensity to ingest toxic foreign materials despite the availability of adequate forage. It is worth noting here that zinc and lead and their salts have been used in the manufacture of many industrial products used in the construction trades, especially those of older manufacture. Zinc-containing substances are employed in the manufacture of rubber products and in pigments used in paints and linoleum. Examples of lead-containing products are: some paints, ashes from lumber painted with lead-containing paints, and old window putty and caulk, to name a few.

In June three crossbred cows were found dead within a short period of time on a central Arizona ranch. The only consistent lesions were congestion of the lungs and some scattered hemorrhages in the myocardium. The cow that was presented to the AzVDL had an udder with a good quantity of milk and the rumen contained normal appearing ingesta. So-called **salt poisoning** (sodium ion toxicity) was confirmed by finding an elevated brain sodium level of 2099 ppm. The poisoning was apparently precipitated by moving cows from an area where salt was relatively inaccessible to an area where abundant loose granular salt was provided. Water was readily available to the cattle and so water deprivation was not considered to be a factor. This case is presented due to concern that this may be a much more common condition in the southwest than we readily recognize because the disease is essentially lesionless in cattle. Also, salt-deprived cattle may over consume loose supplements when they suddenly gain access to them. Following the initial diagnosis, further investigation of the problem was initiated. Three apparently unaffected cows were selected at random and urine samples were collected and analyzed for sodium. Two of the three cows were found to have very low urine sodium levels (0.56 and 2.3 mEq/l; normal range 8-40 mEq/l), suggesting salt deficiency. The sodium concentration was very low (0.02-0.03%; adequate 0.18 - 0.67%) in five major range forage plant species available to the cattle. In addition the ranch is home to a large number of resident elk, which share the pastures much of the year with the cattle. The ranch manager reported that the elk would actively compete with the cattle for the loose salt when it

was available. This probably increased the rate of consumption and possibly decreased the longer term availability of the salt. One might imagine a scenario in which salt deficient cattle are not getting enough salt supplement to meet their requirements due to competition for the limited supplements. A few animals which suddenly gain access to newly distributed salt supplement then over-consume the loose product, and die of salt poisoning. After the diagnosis was made the ranch switched to hard salt blocks, which decrease the rate of consumption. The ranch has reported no further death losses.

Mycotic rumenitis, hepatitis and enteritis was the cause of death in a two-year-old Holstein presented for bloody diarrhea and harsh lung sounds. Rumen acidosis damaged the integrity of the rumen epithelium and with the alteration in the normal flora, allowed opportunistic fungi to invade ruminal blood vessels and spread to the liver.

Equine



An unusual case involved a twelve-year-old Arabian-cross mare. The animal had a history of neurologic problems with progression to seizures. The early signs included a stiff neck and dropped shoulder for the first one to two weeks. This was followed by postural problems and then left sided paralysis and circling. A large **epidermal inclusion cyst** was found within the brain at necropsy. The lesion was in the right cerebral hemisphere and consisted of a 4-5 cm diameter rounded lesion centered above the thalamus. The lesion included an amorphous yellow central mass that had a friable consistency. It was surrounded by areas that could be identified as being somewhat capsular or fibrotic. Histopathology identified an epithelial lining within the cystic tissue that surrounded the amorphous material. This epidermal inclusion cyst was probably developing throughout the animal's life and merely reached a size where the horse became unable to compensate.

Small Ruminants



Nutritional myopathy ("White muscle disease") was diagnosed in a four-month-old ram lamb weighing about 60 lbs. The flock had three downer lambs and several that had been "knuckling over" in the hindlegs. At necropsy, most of the femoral musculature of the hindlegs was discolored white. Pale streaking was also evident in the iliopsoas musculature. Microscopic examination of sections of the affected musculature revealed lesions typical of nutritional myopathy. The lambs were fed alfalfa hay and a grain-cottonseed meal-molasses-salt creep mix with no added selenium. Typically the lambs in this operation receive an injectable selenium product but the herdsman thought that the injection may have been omitted in the case of the affected lambs.



Avian

Polyomavirus infection was the cause of sudden death in a six-week-old Moluccan cockatoo. At necropsy, the only gross lesions were petechial hemorrhages on the epicardium. Characteristic intranuclear inclusion bodies were visualized microscopically in cells of the pulmonary interstitium, hepatocytes, bile duct, spleen and heart.

Megabacteriosis was the putative cause of chronic weight loss, diarrhea and death in four budgerigars. Megabacteria are of uncertain taxonomy. They are very large, septate organisms which arrange themselves in large bundles in proventricular glands of birds. The clinical syndrome “going light” in budgerigars has been attributed to heavy infection with this organism. However, as yet, Koch’s postulates have not been successfully fulfilled and small numbers of the organism can be found in the proventriculus of “normal birds.” There are skeptics who are not convinced of a causal relationship.

Proventricular dilatation syndrome (PDS) was diagnosed at necropsy in a 22-week-old psittacine of unspecified type. The crop was full of ingesta and lesions included a proventriculus that had a thin, translucent wall and was greatly distended with ingesta. Microscopic examination of the proventriculus revealed perivascular infiltrates of mixed leukocytes in tunica muscularis. Sparse lymphoplasmacytic perineural leukocytic infiltrates were also present. The cause of PDS is unknown although there is some reported evidence that suggests an infectious agent. This remains unproven. Prognosis is generally poor for affected birds.

Trichomoniasis was diagnosed in a three-month-old conure and a budgerigar of unspecified age. Both had pharyngeal lesions typical of trichomoniasis. A swab of the lesions from the budgie was culture-positive for trichomonads. Trichomoniasis, although generally thought of as a disease of columbid birds (i.e. pigeons, dove), has a fairly wide host range and has been seen in wild passerine birds (i.e. finches) in the Tucson area. Cross-infection between species can be expected in mixed-species aviaries or when infected wild +birds gain access to food and water utilized by captive birds.



Canine

An eleven-week-old, male Great Dane puppy presented clinically with apnea and cyanosis. The heart rate and pulse were abnormal. There was a heart block and a grade 3 to 4 out of 6 holosystolic murmur.

Aortic stenosis was diagnosed at necropsy. The left ventricle was hypertrophied. The ventricular chamber was dilated. A prominent, subvalvular stenotic ring was subjacent to the aortic semilunar valve.

Parvovirus myocarditis was diagnosed in a three-week-old female Boxer puppy. This was one of six puppies from a litter to die suddenly. Histologically, the lesions consisted of multifocal myocardial necrosis, interstitial edema and intranuclear inclusion bodies in cardiac myocytes. There were no intestinal lesions. Acute parvovirus myocarditis occurs only in very young puppies (less than 6 weeks usually) because the virus requires an actively dividing cell population for replication. Typically there are no intestinal signs or lesions. Sudden death is the typical presentation. The syndrome was more common when parvovirus first appeared in this country but dissipated, supposedly because of widespread vaccination of dams.

A four-month-old Rottweiler-mix puppy died following a four day illness characterized by erratic behavior, lethargy and nervous involvement of the head. There was a subcutaneous swelling in the throat area. At necropsy, there was subcutaneous edema of the intermandibular space and ventral cervical areas. The esophagus was slightly dilated. A 4 cm, tan mass was in the hilar area above the lung pressing on the esophagus. On cut surface, the mass exuded creamy, greenish exudate. Scattered 0.25-0.5 cm, tan nodules containing similar exudate were present in the liver, kidneys and throughout the brain. Histologically, the exudate containing nodules represented pyogranulomas containing large numbers of gram positive, branching, filamentous bacteria compatible with *Nocardia* sp. **Canine distemper** virus infection was identified by the presence of typical inclusion bodies in the epithelium of the urinary bladder, inclusion bodies and bronchointerstitial pneumonia in the lungs, and inclusion bodies in glial cells of the brain. Immune suppression by the viral infection leading to a secondary opportunistic infection with *Nocardia* followed by multisystemic bacterial dissemination was the likely pathogenesis. The large hilar pyogranuloma resulted in intermandibular and ventral cervical edema by compromising venous and lymphatic circulation.

Canine distemper was diagnosed at necropsy in a six-month-old mixed canine weighing 40 lbs. The dog developed seizures about three to four days prior to its death. The submitting veterinarian reported that the dog had an uncertain vaccination history. At necropsy stomach and intestinal mucosae were hemorrhagic as was the endocardium of the left ventricle. Microscopic examination revealed changes typical of canine distemper, including pale pink-to-orange intranuclear inclusions in glial cells of brain compatible with those of distemper virus infection. It was felt that the gastrointestinal hemorrhage was the result of shock (i.e. “shock gut”)

Hypoadrenocorticism (Idiopathic Adrenocortical Atrophy) was diagnosed as the probable cause of death in a nine-year-old female German Shepherd found dead. Prior to death, the dog had been vomiting. The adrenal

glands were not found at necropsy and severe adrenocortical atrophy was presumed. The spleen was engorged with blood. Clinical chemistry workups were not performed so there was an absence of supporting laboratory data. Development of clinical signs of hypoadrenocorticism can be insidious and likely hyperkalemia occurred and impaired circulatory function to the point that circulatory collapse occurred.



Feline

Tyzzler's disease was the cause of necrotizing enteritis in a six-week-old Persian-cross kitten received for necropsy. This was one of three sick kittens from a cattery. The clinical signs included generalized muscle flaccidity, dilated pupils and spasmodic clenching of the jaws. Histologic examination of the small intestine revealed multifocal, suppurative and necrotizing enteritis. Epithelial cells on villi contained bundles of filamentous, silver positive bacteria consistent with *Bacillus piliformis*.

Cerebral lymphoma was diagnosed in a ten-month-old feline received for necropsy. The submitting veterinarian noted that the "cat died suddenly after two seizures". Microscopic examination revealed prominent lymphocytic cuffing of cerebral blood vessels. Lymphocytic infiltration of neuropil surrounding the most severely affected vessels with resultant neuronal necrosis was also evident. Imprints of spleen and bone marrow were negative for feline leukemia virus.

Hypertrophic cardiomyopathy was diagnosed in a seven to eight-year-old male longhair domestic feline weighing 15 lbs. The neighbor found the cat dead in the yard. No prior clinical signs were noted. The cause of hypertrophic cardiomyopathy in cats is unknown. One reference reports the mean age of onset is reported to be 4.8-7 years, with neutered males at greater risk than neutered females. Sudden death is one of the possible sequelae.

A fourteen-year-old neutered male domestic shorthair feline was presented with a history of previous cryptococcal lesions affecting the subcutis of the thorax. Clinical disease progressed to posterior paresis then quadripareis with intermittent opisthotonus. Necropsy findings showed some accentuated liver lobular patterns. There was some mucinous material on the surface of the brain but this could not be differentiated from autolysis because the carcass had been frozen. *Cryptococcus neoformans* can be found in animals where there is a tendency for this organism to affect the meninges. Ocular lesions are not unusual. In this case it caused the mucinous appearance of the meninges. Some *Cryptococcus neoformans* organisms were associated with connective tissue around the optic nerve. Systemic mycoses are a sporadic finding and are somewhat unpredictable. Diagnosis can be supported by serology, skin tests, culture and histopathology.



Wildlife

Colibacillosis produced by a beta hemolytic, enterotoxigenic *E. coli* was the cause of gastroenteritis and septicemia with heavy mortality in a group of captive Black-footed ferrets. The isolates were non-typable by the Kauffman scheme of serotype definition based on the "O" or somatic and "H" or flagellar antigens, but all of them produced heat stable enterotoxins STa and STb. Both of these toxins are occasionally present in human strains of diarrheagenic, enterotoxigenic *E. coli*; STb is usually produced by the porcine strains. The enterotoxigenic *E. coli* was also isolated from the prepared feed and it is believed to originate in the uncooked, ground rabbit meat used in the formulation of the feed although unmixed meat was no longer available for culture.

Three cases of **avian pox** were diagnosed; a crow, a loggerhead shrike and two cases in wild birds. The crow had crusty scabs on the skin over much of the body with feather loss. The shrike had a severe, proliferative, crusting blepharitis. Lesions were also found in the tracheal mucosa. The third case involved five-week-old ostrich chicks from an ostrich ranch. All had multiple, crusting, raw nodules in the skin of the face, eyelids and maxilla.

Severe intravascular **microfilaremia** causing massive hepatic and myocardial necrosis was the cause of death in a Mourning dove. The person presenting the bird had noticed several dead doves at their Tucson residence. The taxonomy of this parasite is unknown. Many species of birds have similar parasites but most are clinically inapparent. However, the sparrow is known to experience fatal illness due to microfilarial infection. It is unknown if infections are due to many or few parasite species or if the infection crosses species lines. An insect vector is presumed.

A 1 ½-year-old bobcat was found dead at a rehab facility. At necropsy, a spent rifle bullet was found in the lumen of the stomach, presumably ingested with a meal of game-meat. The liver contained 9.84 ppm lead confirming a diagnosis of **lead poisoning**.

Trichomoniasis was diagnosed in an immature American kestrel being treated by a veterinarian for the disease. Moderate emaciation was evident. Despite treatment with oral Flagyl®, the bird died. A typical oral lesion was culture-positive for the organism.



Exotics

Cryptosporidiosis was the cause of wasting, regurgitation and diarrhea in a colony of Leopard geckos.

Inclusion body disease of Boids was diagnosed in a Log boa that had been sick with an “upper respiratory infection for the past month”. Typical inclusion bodies were found in respiratory epithelium of lung, tracheal mucosa, and collecting tubule epithelium of kidney. A localized necrotizing colitis, possibly due to immunosuppression, was also present.

An eighteen-year-old Yellow Collar macaw was presented with a history of paralytic problems and seizures. The bird was in generally good condition and no significant lesions were observed at necropsy. However, histopathology

revealed an **encephalomalacia** in the cerebellar peduncles and the cerebellum that was quite well advanced. Lesions involved the white matter. There was an associated arteriosclerosis affecting meningeal vessels. It is postulated that the vascular lesions caused restricted blood supply to the affected areas of the brain.

*compiled by; Greg Bradley, Robert Glock, T. H. Noon,
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Comments on Diagnostic Update can be directed to
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Diagnosis of botulism

Botulism due to *Clostridium botulinum* type C was diagnosed in three different episodes of mortality in migratory ducks. One case affected ducks in a municipal wetlands project; one affected waterfowl in a waste water reclamation pond, and the site of the third case was not reported. The infection was confirmed in all three cases by the detection of the toxin from the serum of the affected birds by means of mouse inoculation, and identified as type C by a serum neutralization test. **Botulism** was also the tentative diagnosis in the deaths of twelve dairy cows in Central Arizona. The cows were weak and recumbent with signs of paresis. They died a few hours after “going down”. They had been fed alfalfa hay which contained the desiccated carcasses of rodents and small ruminants. A cell-free, filtered extract of one of the small bones found in the hay was lethal for mice, producing the flaccid paralysis and abdominal breathing typical of botulism intoxication. Unfortunately, there was not enough sample for the toxin neutralization test.

Type C Botulism is a common warm weather disease in wild waterfowl in the Western USA, often diagnosed by the observation of flaccid paralysis (“limberneck”) in affected animals. Spores of *C. Botulinum* type C are common in wetlands and many botulism outbreaks occur on the same wetlands year after year. Regular surveillance of affected areas for bird carcasses is recommended.

Preventive carcass cleanup reduces sources of botulism toxin by eliminating saprophytic fly maggots that would infest the carcasses, accumulate the toxin, and then be eaten by feeding waterfowl, thus propagating the disease. When a laboratory confirmation is necessary, the toxin can be detected with relative ease from serum of sick or recently dead waterfowl by inoculation of mice or, more recently, by an ELISA test. Submit serum or whole blood from affected waterfowl or, if not available, crop content or whole carcasses for necropsy.

Bovine botulism (usually caused by *Cl. botulinum* types C or D) is a rare condition, produced by the ingestion of hay contaminated with toxin-laden carcasses of birds or small mammals. The diagnosis is difficult, often based on ruling out other causes of death and the detection of pre-formed toxin in the contaminating carcasses. It is virtually impossible to detect the toxin in the sera of affected cows, but it can sometimes be isolated from the rumen content. Botulism has also been diagnosed in horses (types C, D or E), apparently following the multiplication of the clostridium in necrotic areas of the intestine. Confirmation of the diagnosis in affected horses depends on the identification of preformed toxin or of *Cl. botulinum* from stomach and/or colon contents. Call the laboratory at (520)621-2356 to discuss the selection of samples to submit in specific cases of suspected botulism.

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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