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A Quarterly Publication of the **Arizona Veterinary Diagnostic Laboratory**

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## From the Director:

We welcome Dr. Peder Cuneo who has accepted the position of Extension Veterinarian for the University of Arizona Extension Program. This position has been vacant since the retirement of Dr. Ed Bicknell in December 1998. Dr. Cuneo received his D.V.M. and M.S. from the University of California. He is a diplomate of the American Board of Veterinary Practitioners with a Beef Cattle Specialty. He has completed an in-depth Beef Cattle Production Management series at the University of Nebraska. Dr. Cuneo has extensive practice experience in California and has served as Farm Animal Clinical Veterinarian and Animal Care Specialist at the University of Arizona for the past twelve years. He is now a member of the faculty of the Department of Veterinary Science and Microbiology with an office in the Diagnostic Laboratory. We are enthusiastic about the additional expertise Dr. Cuneo will provide. His major challenge will be to find ways to help improve our outreach and field research capabilities. Dr. Cuneo can be reached at 520-621-2356 or e-mailed at [cuneo@u.arizona.edu](mailto:cuneo@u.arizona.edu).

*Robert D. Glock, Director*

## Feature Article:

### **Does *Ehrlichia canis* cause clonal transformation of lymphocytes?**

This is the question Dr. Anne Avery at Colorado State University is asking. She needs the help of Arizona veterinarians to determine the answer. As veterinarians in the Southwest know, *Ehrlichia canis* infection results in a number of interesting and as yet unexplained immunological abnormalities. These include the development of a monoclonal gammopathy, expansion of large granular lymphocytes in the peripheral blood, autoimmune destruction of platelets and perhaps red blood cells and, in the chronic stages, pancytopenia with a loss of precursors in the marrow. Dr. Avery's lab has recently developed a PCR based assay that allows the detection, at high sensitivity and specificity, of clonally expanded lymphocyte populations. This test is now routinely used for the detection of lymphoma in cases where cytology and histopathology results are ambiguous. Using this assay, they have found that a large percentage of dogs with serologic evidence of Ehrlichia exposure also have clonal expansions of T lymphocytes. Some of these dogs were tested because the veterinarian suspected concurrent lymphoma, but some were dogs solicited on the basis of a positive Ehrlichia titer alone. Combining these preliminary results with the clinical observations above, they hypothesize that Ehrlichia infection, through an as yet undescribed mechanism, causes a possibly pre-neoplastic clonal transformation of lymphocytes in a subset of patients. Manifestations of this clonal transformation include monoclonal gammopathy, the expansion of large granular lymphocytes, and the PCR evidence of clonal lymphocyte expansion. Such transformed lymphocytes may undergo further transforming events to become neoplastic, or may resolve with time. In order to test this hypothesis Dr. Avery's lab would like to solicit blood and/or bone marrow from dogs with serologic evidence of Ehrlichia infection, regardless of their hematopoietic status or the chronicity of their infection. She is especially interested in dogs with very high persistent titers and any dogs that develop a monoclonal gammopathy. If you would like to participate, contact Dr. Sharon Dial at 520-621-2356.

## Short Comment Section

The Arizona Veterinary Medical Association recently sponsored a seminar on equine protozoal myeloencephalitis. Dr. David Granstrom, the speaker, is a well-known authority. He stressed the diagnostic importance of a thorough clinical evaluation supported by detection of antibodies to *Sarcocystis neurona* in serum or cerebrospinal fluid. Lesions may be difficult to find in some animals but can usually be identified, particularly in more severe cases. We have not had a submission of tissues

### ***In this issue:***

From the Director	page 1
Feature Article	page 1
Short Comment Section	page 1
Diagnostic Update for Sept, Oct, Nov	page 2
Animal Health Alert	Insert

from a positive case, although we understand that the condition is observed with some frequency in Arizona. We would like to receive several typical advanced cases for necropsy at no charge. Our purpose is to try to confirm that the lesions are typical of those described elsewhere and, more importantly, to attempt to determine if the causative organism, *Sarcocystis neurona*, is any different from the one typically described. One objective would be to try to begin to determine the primary host in this area where the known primary host, the opossum, is scarce or absent. Please call Dr. Robert Glock at 520-621-2356 if you believe you have a suitable case for submission.

## Diagnostic Update

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

### Bovine



***Escherichia coli* septicemia** in six-day-old Jersey calves at a dairy was traced to a contaminated well on the dairy. An unusual source of infection was suspected since more than one type of isolate was involved. The well cap had been damaged and not repaired. Run-off from the dairy after recent rains flowed downhill into the well.

A one-and-one-half year old Holstein heifer was reported to have had numerous discolored areas in the apex and papillary muscles of the heart. This was accompanied by severe congestion of the liver and lung. Numerous sarcocysts, presumed to be *Sarcocystis cruzi*, were associated with numerous lesions consisting of eosinophilic inflammatory change and myodegeneration. The lesions suggest that **sarcocystosis** may have been the cause of death. Sarcocysts are frequently present in bovine muscle but usually in much lower numbers and with no associated inflammation. Attempts to prevent infections with *Sarcocystis* sp. as well as *Neospora caninum* should include efforts to prevent carnivore feces, particularly dog feces, from contaminating live-stock feeds.

***Neospora caninum*** infection was the diagnosis in a five-month bovine fetus received from a dairy herd. Microscopic lesions in the heart were typical of *Neospora* infections, but immunohistochemistry was negative. The diagnosis is considered appropriate in a herd with typical lesions and prior positive identification of *Neospora* infections even without specific staining.

**Ruminal tympany (bloat)** was diagnosed in an eight-year-old Simmental cross cow weighing 1200 pounds. The owner

had noted the cow to be “down last night, apparent left leg injury”. At necropsy the carcass was bloated. Microscopic examination of sections revealed an acute pneumonia of mild to moderate intensity. Toxicology screening revealed an excessive level of sodium in brain tissue (2,043 ppm; adequate range 800-1400 ppm; toxic range 1800-2400 ppm w.w.) The value was confirmed in duplicate and an additional diagnosis of **sodium ion toxicity (salt poisoning)** was rendered. Sodium ion poisoning can occur as a result of salt deprivation followed by over-consumption of salt, or from water deprivation.

**Probable salmonellosis, probable copper toxicity, and vitamin A and E deficiency** were diagnosed in three moribund ninety-day-old dairy heifers. All three calves were emaciated and had considerable fecal soiling of the perineum and tail. Necropsy revealed the heifers to be emaciated and subcutis was discolored yellowish in two of the calves. The contents of the cecum and colon in all three calves were liquid. Moderate to heavy growths of group B *Salmonella* organisms were isolated in cultures of lung, gall bladder, and gut. In addition, excessive levels of copper in liver tissue were found in all three. Vitamin E levels were non-detectable in serum from all three calves. Vitamin A was non-detectable in serum of one of the calves. Young calves are reported to be more susceptible to copper toxicity than older cattle. The icterus seen in two of the calves may have been the result of chronic copper toxicity. Icterus is reported to occur in copper toxicity in cattle along with depression and anorexia.

### Porcine



**Pneumonia** with accompanying pericarditis and epicarditis was diagnosed in a three-week-old Hampshire gilt that died after an illness of one day duration. The necropsy examination revealed consolidation and dark purple discoloration of the apical and cardiac lobes of both lungs and roughening of the pericardial sac and epicardium due to fibrinous adhesions. Cultures of lung tissue yielded moderate growths of alpha hemolytic streptococci.

### Small Ruminants



We received two four-month-old lambs. ***Pasteurella hemolytica*** was the cause of acute pneumonia and reported sudden death in one. *Pasteurella* pneumonia in lambs seems to develop more rapidly than in calves. The second lamb was a poor doer and had chronic **proliferative enteritis**. This condition is common in pigs and hamsters and is caused by infection with *Lawsonia intra-cellularis*. The organism was present in this lamb but etiologic significance is unknown in species such as lambs and foals where the condition occurs very sporadically.

## Avian



**Avian malaria** and **avian pox** were found in a grackle collected from the grounds of a zoo.

**Chlamydial infection (Psittacosis)** was found in a four-month-old conure. The bird had a history of lethargy. Regurgitation had also been observed for the last several days and is suspected to have been caused by advanced inflammatory lesions in the crop due to **Candidiasis**.

A four-year-old parrotlet had numerous granulomas in the liver, ovary, and intestine. **Avian tuberculosis** was diagnosed on the basis of numerous acid-fast organisms in the tissues.

**Hemosiderosis** was diagnosed in a one-and-a-half-year-old male conure that had been ill for three days. Clinical signs were not specified. Prominent accumulations of iron-positive pigment were present in the cytoplasm of hepatocytes and analysis of formalinized liver tissue revealed an excessive amount of iron (1967 ppm; normal ~ 300 ppm).

**Severe nematodiasis** was diagnosed in a six-week-old peacock. The bird was euthanized for diagnostic purposes and six other birds had died in the previous week. The small intestinal lumen contained many ascarid-type nematodes.

**Nematodiasis** of the mucosa of the ventriculus was diagnosed in a five-year-old female Red cheek Cordon Bleu finch (Waxbill) that died after an illness of two days duration. The owner noted, "slow onset of listlessness". Microscopic examination of the gizzard revealed several nematode cross sections underneath the koilin layer. Visceral gout with accompanying nephrosis was noted in the kidney.

**Avian tuberculosis** was diagnosed in a Yellow faced parrotlet that had been sick for about one month. The owner noted "shaking and acting neurological". The bird was kept in an outdoor aviary. Microscopic lesions were typical of avian tuberculosis in liver and in small intestine. Avian tuberculosis is a low hazard zoonotic disease.

## Feline



We received a seven-month-old male Bengal cat with a history of anorexia, lethargy, and upper respiratory signs. A large amount of gelatinous fluid was found in the thorax and abdomen. Inflammatory lesions observed microscopically were typical of **feline infectious peritonitis**. This disease is sometimes difficult to identify antemortem.

A sixteen-year-old cat with intractable diarrhea had multicentric **biliary cystadenomas**. These were accompanied by diffuse chronic cholangitis.

Acute **pancreatic necrosis** was the cause of death in a very obese three-and-a-half year old female domestic cat.

**Ethylene glycol intoxication** was diagnosed in a two-year-old female Siamese. Two other cats in the household had also died. The cat that was presented for necropsy became weak, staggered, and developed convulsions prior to death. Prior to its death, the cat had appeared to be healthy. Necropsy revealed the stomach lumen to be empty except for a small amount of mucus. Microscopic examination of sections of kidney revealed the lumens of most renal tubules to be filled with clusters of crystals that were birefringent under polarized light. A cluster of birefringent crystals was also found in neuropil of the cerebrum. Ethylene glycol was detected in urine from the cat by GC/MS.

**Possible trauma** was diagnosed in a kitten that had been noted to be "spinning and flipping" when restrained. The cat went progressively "downhill" and had a subnormal temperature. At necropsy there was locally extensive subcutaneous and intramuscular bruising of the occipital area of the head and the proximal cervical area. Internally, there was locally extensive extradural hemorrhage over the dorsum of the brain. Testing for rabies was negative and it was felt that the intracranial hemorrhage would likely account for the "neurological signs" that were described.

**Stenosis with obstruction of the small intestine** caused by an adenocarcinoma was diagnosed in a six-year-old spayed female Siamese. The animal had been sick for three months and was finally euthanized. Clinical signs included chronic vomiting and weight loss. Necropsy revealed the small intestine to be ballooned and distended with ingesta about twenty-one inches distal to the pylorus. The distension was caused by stenosis with kinking of the intestine. The affected area of intestine was thickened and microscopic examination revealed an adenocarcinoma.

## Canine



An eight-year-old Basset had melena. At necropsy there was blood in the stomach and intestine. A large ulcerated thickening in the pyloric region of the stomach was identified as **adenocarcinoma** of the stomach.

**Streptococcal pneumonia** was the cause of death in a five-week-old Mastiff puppy presented with a history of sneezing and mucous drainage from the nose. Surviving puppies in the litter had raspy lung sounds. Group G *Streptococcus*

sp. was recovered from the lungs.

Two cases of puppies **choking** on kibbled dog food were seen during this quarter. One was a seven-week-old male Boxer and the other an eight-week-old English bulldog. Neither of the owners observed the death of their puppy. Both had been active and healthy just prior to being found dead. In each case, the size of the kibble was just small enough to be aspirated through the rima glottidis into the trachea but apparently too large to be expelled again. The kibble acted as a ball valve allowing inspiration but not expiration. This resulted in the notable gross necropsy finding of hyperinflated lungs in both cases. If the size of the kibble had been larger or smaller, it is likely that neither puppy would have expired.

A three-year-old male hound died shortly after presentation to the veterinarian for the sudden onset of ascites. The urine specific gravity was 1.002. At necropsy, the dog's abdomen was filled with clear amber fluid. The kidneys were of normal size but were pale and had occasional cortical pitting. A triangular, plum-colored infarct was present in the left apical lung lobe. Severe **glomerular amyloidosis** was found on histologic evaluation of the kidneys.

A **cerebral abscess** caused by a **migrating grass awn** was the cause of seizures and death in an eighteen-week-old, female Basset mix puppy. The abscess was located in the left temporal area of the cortex. In addition to the grass awn, scattered colonies of bacteria were microscopically visualized in the abscess. *Pasteurella* sp. was isolated.

We received a five-month-old German Shepard with a history of sudden death. The cause of death was severe **congenital renal dysplasia**. The interesting feature of this case is the lack of a history of long-term clinical illness.

Severe **hemorrhagic gastroenteritis** in a four-year-old Rottweiler was associated with the presence of heavy populations of *E. coli* in the intestine and mesenteric lymph nodes. Advanced renal amyloidosis may have been a predisposing factor in the debilitation of the animal.

An eleven-year-old male Bull terrier died after a period of illness associated with an enlarged prostate gland. A malignant **neuroendocrine tumor (chemodectoma)** of the heart base with metastases to lung, liver, and thyroid gland was identified. This dog also had a seminoma of the right testicle and a Leydig cell tumor of the left testicle. The enlarged prostate gland was hyperplastic but not neoplastic.

**Metastatic osteosarcoma** was diagnosed in a seven-year-old spayed female Rottweiler. A previous diagnosis of osteosarcoma in the left proximal humerus had been made in October of 1999 and treated with amputation of the affected leg plus chemotherapy. Regional lymph node me-

tastasis was noted microscopically at the time. At necropsy, numerous metastatic tumorous lesions were visible in mesentery, gut wall, parenchyma, spleen, kidneys, and in all lobes of both lungs.

**Undifferentiated carcinoma** of the adrenal gland and kidney was found in a thirteen-year-old neutered female Labrador canine that had been diagnosed in September of 1999 as having an osteosarcoma in the left distal radius. The animal was treated with palliative radiation and chemotherapy. At necropsy, the adrenal glands were severely effaced by neoplastic cell growth and a tumor focus was also found in the cortex of one kidney. The extreme degree of anaplasia made a definitive morphologic classification of the neoplasm impossible.

**Coccidiosis** was diagnosed in a nine-week-old male Pitbull puppy weighing approximately six pounds. The puppy had been presented to the submitting veterinarian for weight loss, anorexia, and watery diarrhea. Neurologic signs were noted four days later. At necropsy, there was locally extensive subcutaneous edema in the submandibular area. Microscopically, essentially all sections of gut contained numerous organisms with morphology compatible with that of coccidia.

**Obstruction** of the pylorus and upper duodenum was diagnosed in a male adult Greyhound. The dog had been presented for weakness, vomiting, and bloody diarrhea. At necropsy, the pyloric and proximal duodenal lumens were obstructed by a dense mass of plastic, fibrous material that contained fairly large, sharp angular pieces of clear plastic. The mucosa of the small intestine around and distal to the obstruction was discolored dark red and appeared to be hemorrhagic.

**Severe subacute to chronic endocarditis** of the left atrioventricular valve due to a gram-positive bacterial infection with secondary embolization to the interventricular myocardium and spleen was diagnosed in a two-year, four-month old neutered male Great Dane. The dog died after an illness of five days duration and prior to the illness had been noted to be "very healthy". Clinical signs were described by the owner as "breathing heavily, drooling and droopy, mucousy eyes". The dog was also unable to stand and had diarrhea. At necropsy, the lung parenchyma was wet and exuded fluid when incised and the tracheal lumen was full of froth. There was an increased amount of fluid in the pericardial sac and a section of the heart revealed brownish, verrucous, proliferative lesions on the dorsal aspects of the valve leaflets of the left atrioventricular valve, along with a locally extensive area of pale discoloration of the intraventricular septum of heart. Several nodular lesions were scattered throughout the parenchyma of the spleen. Microscopic examination revealed the septal lesion of the heart to be an area of myocardial necrosis and the splenic lesions to be infarcts due to vascular thrombosis. Gram-staining revealed a few

colonies of gram-positive cocci organisms in sections of the spleen and within the valvular lesions of the heart.

**Strychnine intoxication** was diagnosed in a six-year-old female Retriever mix which had been presented to an emergency animal clinic for treatment. At necropsy, the stomach contained mixed ingesta that contained a few chunks of meat-type material along with milo-type grain kernels that had a greenish discoloration. The stomach contents were positive for strychnine.

**Intoxication with brodifacoum** was diagnosed in a one-year-old female Jack Russell terrier that had been treated for a swelling of the mandibular area that was suspected to have been an infection. The dog had been treated three days previously for "cactus in mouth". The dog died suddenly following treatment for the submandibular swelling. Necropsy revealed prominent swelling of the ventral aspect of the neck and submandibular area caused by locally extensive subcutaneous hemorrhage which extended along the ventral aspect of the neck and into the thoracic cavity via the thoracic inlet. Intrathoracic hemorrhage was also extensive and filled the mediastinum and pericardial sac. Anticoagulant rodenticide poisoning was suspected and testing revealed an unusually high level (1,190 ppb) of the coumarin anticoagulant, brodifacoum, in liver tissue.

**Malignant melanoma, metastatic to lungs**, was diagnosed in an eight-year-old spayed female canine that died after an illness of one-to-two days duration. The owner noted a decreased appetite the night before the animal's death. The animal was very weak and died the following day. At necropsy, there were numerous, variably sized nodular, tumor foci that were often pigmented scattered throughout all lobes of both lungs. Adjacent pulmonary parenchyma was considerably reduced and atelectatic. The tumor masses varied from a few millimeters in size to about two-to-three centimeters in diameter. Microscopic morphology was typical of malignant melanoma.

## Exotics



**Canine distemper virus** infection was the cause of death in a six-week-old raccoon. The primary gross necropsy findings were emaciation and dark-red, firm lungs.

Severe **renal gout** was identified in a Soshow chameleon. The cause is unknown but gout is often associated with nutritional causes.

**Pneumonia** due to *Staphylococcus aureus* infection was diagnosed in a two-year-old female chinchilla that died under anesthesia. Gross and microscopic lesions were typical and bacterial cultures of lung tissue yielded moderate to heavy growths of *Staphylococcus aureus*.

## Wildlife



Prairie dogs from several different sources had multiple necrotic foci and microabscesses in multiple organs. *Staphylococcus aureus* was identified as the cause of septicemia.

**Colibacillosis and cryptosporidiosis** were diagnosed in a juvenile javelina that developed diarrhea and lethargy four weeks after entering a rehabilitation facility. This was the second javelina to die with these signs.

## Zoo



**Hepatocellular and renal tubular hemosiderosis** was diagnosed in a seven-year-old female Grosbeak from an Arizona zoo. Microscopic examination of sections of liver and kidney revealed prominent cytoplasmic accumulations of iron-positive pigment in the cytoplasm of hepatocytes and in renal tubular epithelial cells. Analysis of formalinized liver tissue revealed an excessive amount of iron (4330 ppm; normal ~ 300 ppm).

**Hemosiderosis** was diagnosed in a White chested mynah from an Arizona zoo. Microscopic examination of sections of liver revealed prominent accumulations of iron-positive pigment in the cytoplasm of essentially all hepatocytes and most Kupffer cells. Analysis of formalinized liver tissue revealed an excessive amount of iron (8341 ppm iron; normal ~ 300 ppm).

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

*compiled by Greg Bradley, Bob Glock, Carlos Reggiardo, T. H. Noon, Sharon Dial, and Barbara Hiers*

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