



# Newsletter

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## Cooperative Extension and the Arizona Veterinary Diagnostic Laboratory

### From the Director of the AzVDL

The Arizona Department of Health Services has recently issued a plague Advisory for Northern Arizona. A human death from plague was confirmed in Coconino County, and the disease has also been identified in Apache, Coconino, Greenlee and Yavapai counties by way of routine surveillance of coyote, rodent and flea populations.

Feline plague and cases of cat to human transmission of plague have been on the increase in the Southwest. When either pneumonic or bubonic plague is suspected in a cat, a rapid diagnosis by culture or fluorescent antibody test is very important. We have been able to obtain a limited amount of FA conjugate for plague testing from the CDC for the testing of suspicious bacterial isolates from lymph node aspirates, blood samples, swabs (or carcasses). Serological testing can be obtained from the Arizona State Health Laboratory Vector-borne and Zoonotic Diseases section (602-230-5917).

With this issue of the AzVDL Newsletter we are initiating a series of profiles that will briefly describe the different service sections of the laboratory to our clients, including the nature of the tests offered and suggestions for specimen submissions. The Virology laboratory is the first section to be profiled. We will also introduce the different areas of research interest by the faculty of the Department of Veterinary Science and Microbiology. Dr. Michael Riggs' current research interests are briefly described in this issue. To obtain any specific information on the instructional or research programs of the department, the Microvet InfoServer web site can be accessed at <http://microvet.arizona.edu> using Netscape or Internet Explorer.

ascitic fluid. The heart was enlarged with a rounded silhouette due to dilation of the right ventricle and atrium. The right ventricle and branches of the pulmonary arteries contained numerous *Dirofilaria immitis*. The affected arteries had thickened walls and rough intimal surfaces characteristic of the chronic endarteritis seen with **canine heartworm disease**. The owner of this dog was adamant that they had not traveled outside of the Tucson area. She noted, however, that the place where she lived had drainpipes which contained standing water. A heavy mosquito infestation occurred during the preceding year. The Arizona Veterinary Diagnostic Laboratory was established in 1982 and has had an average of 135 canine necropsy submissions annually since then. This is the first case of canine heartworm disease we have confirmed in a native Arizona dog that could not be traced to travel in an endemic area. Our submissions are heavily skewed to southern Arizona. We would be interested in hearing about cases from other areas of the state which have been confirmed by necropsy.

Five cases of suspected **heat stress** related deaths were seen. The dogs ranged in age from two to eight years and were housed outdoors. All were found dead by their owners, usually in mid to late afternoon, without a prior history of clinical illness. Necropsy examination revealed few specific findings other than the absence of pre-existing disease or toxic substances. In general, post-mortem decomposition was further advanced than would be expected for the time frame reported. Pulmonary congestion was sometimes marked. Dehydration, subjectively assessed based on the degree of loss of skin elasticity, dryness of the subcutaneous tissues and recession of the eyes into the orbits varied from minimal to severe.

A ten-year-old, spayed female Poodle was euthanized following acute onset of pulmonary edema which proved refractory to Lasix®. At necropsy, there was a severe, chronic **restrictive pericarditis**. The pericardial sac was red streaked and tightly adherent to the epicardium. Infectious microorganisms were not identified via

### Diagnostic Update

The following is a select sample of cases submitted to the AZVDL during the past quarter.

#### Canine



A six year old Jack Russell terrier died after a brief clinical illness characterized by dyspnea. Gross necropsy examination revealed a pendulous abdomen filled with

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culture or microscopic examination. A bacterial or fungal agent is the usual cause and *Coccidioides immitis* is the most common cause of effusive and restrictive forms of pericarditis seen in necropsy examinations performed at this laboratory.

A five-year-old, castrated male, Maltese died during induction of anesthesia for a routine elective procedure. There was no history of significant pre-existing disease. At necropsy, focal **lymphocytic myocarditis of the atrio-ventricular node** was found. Inflammatory lesions in the conduction system of the heart are rare findings in dogs in general and in unexpected anesthetic deaths especially. The cause is unknown. Presumably, the inflammation, although clinically silent, provides sufficient irritation to induce a dysrhythmia during anesthetic induction. The vast majority of dogs presented to this laboratory with a history of **unexpected death during anesthesia** have no necropsy lesions. Most occur during the induction phase and are presumed to represent **idiosyncratic reactions** to the anesthetic agents. As this case illustration demonstrates, underlying disease, although rarely present, is sometimes a factor in cases of unexpected anesthetic death. Thorough post-mortem examination by a pathologist should be standard in these unfortunate occurrences.

We have received several submissions with a history of "tiny white insects attached to the skin of dogs". These have been identified as nymph stages of *Rhipicephalus sanguineus*. The AZVDL can arrange for identification of arthropods of uncertain taxonomy through the Department of Entomology. The samples should be submitted to the diagnostic laboratory with a history which should include where the insect was found, species of animal infected and geographic region.

A **subvalvular pulmonic stenosis** was diagnosed in a four-week-old Irish setter puppy with a history of cardiac insufficiency. Gross examination of the heart revealed a subvalvular fibrotic ring restricting blood flow through the pulmonary outflow tract leading to the creation of a prominent post-stenotic dilatation of the mainstem pulmonary artery. There was right ventricular and right atrial enlargement and mild hypertrophy of the right ventricular free wall. Pulmonary stenosis is a common congenital defect in the dog. It is an inherited defect in Beagles and is suspected to be so in English bulldogs, Chihuahuas and terrier types. An interesting incidental finding was **agenesis** of the right kidney. The left kidney was normal. This reinforces the observation that where there is one congenital defect, others may be found in other organ systems.

*E. coli* was the cause of severe enteritis in an eight-week-old puppy received for necropsy. The owner, a breeder, had lost several puppies in the last two months.

Two separate cases of **aldicarb (Temik®)** poisoning have been seen in dogs this summer. In both instances, adulterated food was found near the dead animal or in the stomach contents. The clinical signs reported included ataxia, vomiting, and diarrhea. Aldicarb is a highly toxic,

carbamate compound used as an agricultural insecticide. It is a black granular substance that is easy to recognize in bait or stomach contents of poisoned animals. The granules are approximately the size of poppy seeds. Confirmation of aldicarb poisoning is by infrared spectroscopic analysis of the suspect material and can be performed at the AzVDL. If poisoning by this or similar acetylcholinesterase inhibitors is suspected in an animal (live or dead) and bait or stomach contents are not available, the acetylcholinesterase levels in whole blood (purple-top tube) from a live animal or brain tissue from a dead animal would be suggestive of the diagnosis. The preferred tissue for this determination is one half brain.

## Feline



An eight-week-old, female Abyssinian kitten was euthanized following acute onset of vomiting, anorexia and "fading." **Feline panleukopenia** was diagnosed at necropsy.

**Calicivirus** was isolated from conjunctival and throat swabs of an eight-week-old, male, domestic shorthair with a clinical history of fever, depression, generalized stiffness and lameness. Four of five kittens in the litter were affected. The litter had a mild upper respiratory disease one week prior to onset of the current problem. Two strains of feline Calicivirus are recognized as causing a transient, febrile "limping" disorder in kittens. The course of the illness varies from two to four days. One of the two strains is not neutralized by feline Calicivirus vaccine-induced antibody.

**Histoplasmosis** was the cause of a severe, granulomatous pneumonia in a 6 year-old, male-neutered, feline. The animal was presented to a local clinic with abnormal breathing in early June. Radiographs revealed a mild interstitial infiltrate and antibiotic therapy was instituted. In mid-June radiographs revealed a multifocal interstitial pattern in the lungs and examination of a lung aspirate revealed *Histoplasma* organisms. Fluconazole therapy was begun with only minimal improvement and with elevation of liver enzymes the treatment was discontinued. The cat expired a short time after fluconazole therapy had been reestablished. Massive gross lesions were seen in the lungs, and microscopic lesions were present in the lungs, bronchial lymph nodes and spleen. This cat had never been outside the Tucson area.

**Salmonellosis** was the cause of death in a twelve-week-old, female domestic shorthair kitten. There was a two week history of partial anorexia followed by acute onset of ataxia affecting all four limbs. Also present was a horizontal nystagmus with the fast phase to the right. The kitten was dull and demented. Necropsy findings included a chronic active, multifocal enteritis, an abscessed mesenteric lymph node and a severe, diffuse, suppurative meningoencephalitis. A heavy growth of a group B *Salmonella sp.* was recovered from the brain, liver, lung, small intestine, spleen and the abscessed mesenteric lymph node.



## Equine

Sudden death due to a **ruptured aorta** was diagnosed in a sixteen-year-old Thoroughbred stallion. The rupture was a 1.0 cm, three-cornered tear located in the sulcus behind the septal leaflet of the aortic semilunar valve. Blood escaping through the tear dissected between myofibers into the interventricular septum. Hemorrhage into the interventricular septum from an aortic rupture behind the septal leaflet of the aortic semilunar valve is the most common manifestation of the acute aortic rupture syndrome found in equine post-mortem examinations at the AzVDL. It is the most easily overlooked of the three due to the lack of gross hemorrhage into a cavity (pericardial sac or thorax). The other two sites of rupture that may be encountered are more distal in the ascending aorta. Those that occur within the pericardial sac result in pericardial tamponade. Those exterior to the pericardial sac result in hemothorax. This syndrome is restricted to stallions. The sites of rupture are thought to be congenitally weakened sites and elevated blood pressure during exertion (breeding, racing, etc.) is thought to be the precipitating factor. Rupture of the pulmonary artery also occurs in horses but is even more uncommon than aortic rupture.

Severe, fibrinous pleuritis secondary to a **ruptured esophagus** was found in a Quarterhorse gelding. The horse was euthanized following a one week history of fever of unknown origin which failed to respond to therapy. Histologically, the lesion in the esophagus was chronic and fit temporally with the one week history of clinical signs. There was no history of esophageal obstruction or stomach tubing preceding the onset of signs.

## Bovine



**Locoweed poisoning** was the cause of death in a yearling heifer. Six other animals from a herd of fifty were reported "ill" but specific signs were not given. The brain tissue revealed marked cytoplasmic vacuolation in neurons characteristic of swainsonine poisoning. Swainsonine is the toxic principle found in species of *Astragalus* and *Oxytropis* responsible for causing locoism. The toxin inhibits  $\alpha$ -mannosidase, a lysosomal enzyme, resulting in accumulation of a storage product: glycoprotein-derived, mannose-rich oligosaccharides. Locoism is thus an acquired storage disease. Characteristic clinical signs include stumbling, staggering gait, truncal ataxia, muscle tremors, blindness, progressive dullness and wasting.

*Streptococcus sp.* was isolated from the joints of a ten-day-old Holstein calf with chronic **fibrinosuppurative arthritis**. Both carpi were swollen. The joint capsules were thickened and joints distended with turbid yellow fluid containing clumps of yellow/tan fibrinous material.

A group D *Salmonella* was isolated from tissues submitted from a field necropsy of two, four-month-old, Holstein calves. Both calves had lobar pneumonia. The lungs of one of the calves were fluorescent antibody positive for Bovine Respiratory Syncytial virus.

Muscle tissue from a dairy cow which died suddenly was fluorescent antibody positive for *Clostridium septicum*. Histologically, there was severe interstitial edema with gas formation, coagulative necrosis of skeletal muscle fibers and colonization by rod-shaped bacteria consistent with clostridia.

## Avian



**Polyomavirus** infection was the cause of death in a four and one-half-week-old Severe macaw chick. The bird was presented with the complaint that it failed to empty its crop after feeding. Subcutaneous bruising was noted on physical examination. A 10-week-old Eclectus from another aviary was also diagnosed with systemic polyomavirus infection at necropsy. The bird presented to the referring veterinarian with a 5 day history of lethargy and decreased appetite. Hemorrhages in skeletal muscle and liver parenchyma were the prominent gross necropsy findings.

**Psittacine herpesvirus infection (Pacheco's disease)** was diagnosed in a six-month-old, Senegal parrot received for necropsy. Hepatocellular necrosis with typical, intranuclear inclusion bodies were seen histologically and confirmed by fluorescent antibody staining.

## Exotic/Wildlife



A breeder of Bearded dragons lost 10 to 50% of each clutch from an illness characterized by ataxia and head tilt. The illness developed shortly after hatching and continued to one to three months of age. At necropsy, basophilic intranuclear inclusion bodies typical of **Adenovirus** were numerous in many organs including the liver, kidney, small intestine and pancreas. Necrosis of hepatocytes and renal tubular epithelium accompanied the inclusion bodies. No lesions were found in the brain or inner ear.

**Bronchopneumonia** was the cause of death in a male African Rock python. Clinical signs had included labored respirations. *Citrobacter freundii* was isolated.

**Strychnine** poisoning was diagnosed in a wild pigeon. Six other birds died in the area during the preceding week. At necropsy, green-dyed grain kernels were present in the crop.

**Amyloidosis** of the liver, small intestine, adrenal gland and kidney was the cause of death in a swan from a zoo collection. Amyloidosis is common in water birds including swans, duck, geese and flamingos. The cause is unknown.

Three dead pigeons, found in a parking lot, were submitted for necropsy examination. Their crops contained whole kernels of corn. **Avitrol® poisoning** was

diagnosed by mass spectroscopy of the crop contents. Avitrol® (4-aminopyridine) is used as a bird repellent principally in areas where bird accumulation is considered a hazard, or a public health problem. After eating the corn laced with the compound, birds begin to flap wings, vocalize and convulse. It is hoped that this aversive behavior will frighten away other members of the flock. However, it should be noted that birds exhibiting clinical signs of ingestion usually die. There are no lesions in the tissues of affected birds.

Comments on “Diagnostic Update” may be directed to Dr. Greg Bradley by e-mail, [gabrad@ag.arizona.edu](mailto:gabrad@ag.arizona.edu)

### **Profile of the AzVDL Virology Section**

The virology section of the AzVDL provides diagnostic support in the areas of virus isolation, fluorescent antibody (FA) testing of frozen sections and tissue imprints, and serology. The isolation of chlamydia in cell culture is also a function of this section since the techniques required are very similar to those for isolation of viruses.

In addition this section performs the serologic testing of serum samples for 7 common serovars of leptospira using the standard microscopic agglutination test. This requires the maintenance of fastidious, live leptospiral cultures in the laboratory for each serovar.

#### **Techniques Used For Viral And Chlamydial**

**Isolation:** the mainstay of virus isolation is the cultivation of viruses in cell culture. Virus isolation usually requires a start-up time of 4 days to 1 week after initiation of the request in order to prepare specific cell lines for inoculation of test material. First pass cell cultures will generally be held 5-6 days. These are observed daily for cytopathic effects. Total turn-around time will be in the range of 10-14 days provided there are no problems such as contamination of the cultures.

Specific identification of viruses can be made using FA staining of cell monolayers or electron microscopic examination of culture supernatant. A number of FA conjugates are kept in stock for this purpose. Some of these are usable for the rapid identification of viral infection using the frozen tissue section technique as well.

Chlamydia inoculations are generally performed once weekly on Thursdays. Using FA staining, the cultures are examined periodically over the following 7 days for evidence of infection.

The laboratory currently maintains bovine, porcine, equine, canine, and feline-origin cells for the isolation of viruses specific to these animal species along with two general purpose cell lines. Inoculation of chick embryos is occasionally used for the isolation of certain types of viruses (such as influenza and most avian viruses).

**Serology:** the serum neutralization (SN) technique is a sensitive, time-proven standard serologic method that also requires the use of cell cultures. Cell monolayers are inoculated with a mixture of a known test virus and various dilutions of test serum harvested from an animal's

blood sample in order to determine the titer of neutralizing antibody. The presence of antibody indicates that exposure (infection or vaccination) has occurred at some time in the past. Veterinarians are familiar with the usual serology report showing results expressed as numerical antibody titers, but may occasionally receive a result expressed as “toxic”. This indicates that toxic substances present in the serum have destroyed the cell culture and invalidated the test (see “submission tip” below). Substances having this effect are diverse in origin and range from traces of chemical or detergent in the tube to excessive hemolysis or bacterial growth in the serum sample.

In general, detectable antibody to a virus measured in a single sample indicates that exposure (infection or response to vaccination) has taken place at some time in the past, but provides no information as to the time of occurrence of the infection. **As most veterinarians are aware, testing of 2 samples taken 10-14 days apart (acute and convalescent) will provide better information in this regard.**

**Electron microscopy:** this service is primarily used for the identification of enteric viruses (such as Parvo, Rota, and Corona viruses). The frequency of this testing is case-load dependent but is generally performed on about a once-a-week basis.

**Unknown Isolates:** isolates of viral agents that cannot be identified in our laboratory are routinely referred to a federal reference laboratory. Requests for serology not performed here are also routinely referred to an appropriate laboratory. Nowadays, most referral laboratories charge for these services. These charges, at our cost plus shipping, are added to your final bill.

**Miscellaneous Services:** the virology section also provides official serology testing services for Pseudorabies testing in swine using the latex agglutination or serum neutralization tests.

### **Submission Tips**

Blood samples drawn for the purpose of serologic testing should be allowed to clot undisturbed in an inclined position at room temperature for approximately 2 hours. They should not be in direct sunlight. After initial clot retraction, the serum can be poured off into a new serum tube for submission to the laboratory. The serum sample should be kept refrigerated at all times. Serum yield can be increased by spinning down the clotted sample for a few minutes at moderate speed in a centrifuge. The use of “SST” (serum separator tubes) will increase the serum yield substantially but at a greater cost per tube. These also require centrifugation at a specific speed to work properly. In any case, the objective is to provide the laboratory with clean, uncontaminated serum samples that have minimal hemolysis. **In most cases, “paired serum samples” (acute and convalescent sera) will be required for conclusive diagnosis of clinical infections.**

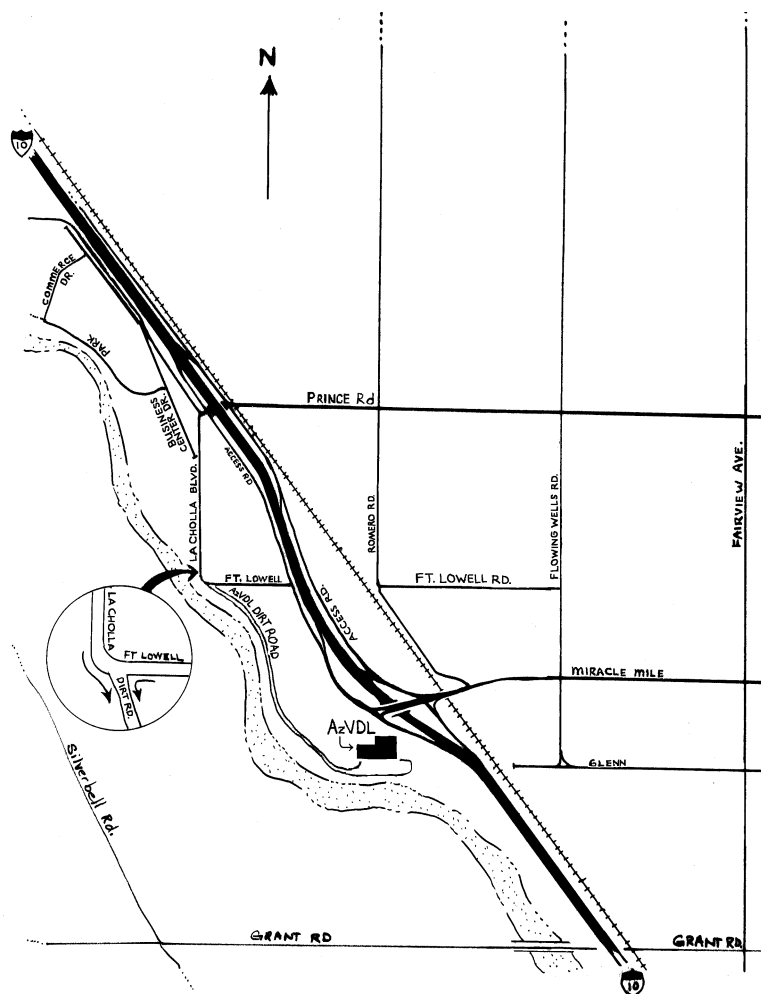
Call ahead for specific instructions if you wish to submit a specimen for viral or chlamydial isolation. In many cases, results can be enhanced if properly collected and timed specimens are submitted in an appropriate transport medium which the lab can send to you. (call 520-621-2356, ask for Dr. Reggiardo). Serum samples submitted in "Venocject" tubes have a high probability of being "toxic" when used in the serum neutralization (SN) serologic procedure. Samples submitted in "Vacutainer" or "Monoject" brand tubes are generally satisfactory.

by T.H. Noon

## Research Profile, Department of Veterinary Science and Microbiology

Michael W. Riggs, DVM, PhD, Diplomate ACVP, Associate Professor

Dr. Riggs' research focus is in immunology and the molecular pathogenesis of parasitic protozoal infections. He currently directs a USDA-supported research program to unravel the pathogenesis of infection by *Cryptosporidium parvum* in calves, and to develop a recombinant vaccine against cryptosporidiosis. Riggs also directs a NIH-supported research program to develop passive immunization against cryptosporidiosis in humans, including those with AIDS and other immunodeficiency disorders. Riggs' remaining research interests are in comparative and veterinary pathology.



### Arizona Veterinary Diagnostic Laboratory

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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