Wyoming State Vet Lab

September 2011 Volume 12, Issue 2

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## MESSAGE FROM THE DIRECTOR

This issue represents my last as Director of the Wyoming State Veterinary Laboratory. I would like to thank all of our clients for your loyalty and support for the lab over the last four years. In turn, we will be here to support you for many years to come. From a more personal standpoint, I look forward to continuing to provide service to you as a pathologist.

The Department of Veterinary Sciences is completing an external nationwide search for a laboratory director and department head. Under the chairmanship of Dr. Todd Cornish, three excellent candidates have been interviewed, the last on August 18 and 19. We hope to be able to announce that this position has been filled in the very near future.

On another personnel matter, Dr. Jeff Adamovicz has been hired as immunologist to replace E.Lee Belden who retired after over thirty years of service to the University. With the hiring of Jeff, some realignment will take place at WSVL. He will serve as the faculty supervisor for the WSVL laboratory sections of regulatory and diagnostic serology, bringing his many years of experience in immunology to these lab sections. I hope you will take advantage of any opportunity to meet and welcome Jeff.

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## INTERESTING CASES FROM WSVL AND OTHER TIDBITS

### *Chlamydophila* in Game-Farm Pheasants

In May, the WSVL was submitted multiple adult pheasants from WGFD's Downar Bird Farm near Torrington. The laboratory isolated *Chlamydophila psittaci*, the cause of psittacosis,

from multiple adult birds. Several faculty members visited the Downar facility along with Dr. Cynthia Tate, WGFD veterinarian and two veterinary students who worked as externs at the WSVL this summer.

Figure 1. Dr. Cynthia Tate (left), WGFD wildlife veterinarian and Colleen Thompson, Kelly Palm Memorial Extern at the Downar Game Farm.

*C. psittaci* is excreted in the feces and nasal discharges of infected birds. It is environmentally labile but remains infectious for a month or more if protected by organic debris (e.g., litter and feces). Some infected birds may appear healthy while shedding the organism intermittently. Shedding can be exacerbated by stress, such as reproduction, rearing of young, relocation, shipping, crowding, and chilling. The disease is transmissible to people. Each year clinical cases of psittacosis occur in people who handle birds or are exposed to their excretions. It is rarely fatal to people, but does cause an unpleasant flu like syndrome.

Dr. Myrna Miller, whose laboratory made the isolations, submitted samples for genotyping to the University of Georgia's Infectious Diseases Laboratory. *C. psittaci* is divided into at least 9 genotypes. Sequence analysis of the outer membrane protein A (*ompA*) gene is currently the best method to classify major genotypes. Each is associated with specific bird groups from which they are predominantly isolated. Genotyping also provides some information on likely virulence. Isolates from Downar were typed as B, which is associated with feral pigeons among other species. Genotype B is generally considered as one of the less virulent genotypes. Little is published on chlamydiosis in game farm species including in pheasants.

To contain the problem and prevent future outbreaks, WGFD personnel had the unpleasant task of depopulating the adult birds. All 1200 pheasants from the farm's brood stock were destroyed, along with chicks and unhatched eggs. The carcasses were incinerated in the WSVL's new medical waste incinerator.

Donal O'Toole, WSVL Pathologist
Myrna Miller, WSVL Virologist

## Unexpected Cardiac Death in Dogs

An intact 7-year-old German shepherd bitch was playing with her owner when she dropped dead. The bitch's dam died under similar circumstances when she was 9 years old. The veterinarian submitted an unfixed heart for examination. Grossly, great vessels and cardiac proportions and weights were unremarkable. There were multiple ill-defined areas of pallor in the myocardium.

Histologically the bitch had distinctive foci of arteriosclerosis affecting intramural coronary arteries. This condition glories in the unhelpful name 'arterial hyalinosis'. In addition to loss of arterial myocytes, there was cardiocyte caliber variation and myocardial fibrosis. Presumably these were the result of hypoxia and probably accounted for death of the dog. We see this entity from time to time in sudden death dogs, as well as an incidental change.

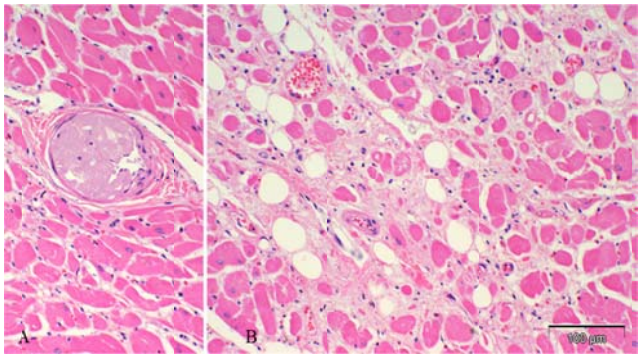


Figure 2. Heart from a dog that died suddenly. A. There is almost complete occlusion of an arterial lumen by amorphous material. B. Marked variation in caliber of cardiocytes, with associated fibrosis.

As is well known, true heart attacks are rare in dogs. This is not just because dogs have limited opportunity to support the fast food industry since, if my dog is anything to go by, they would if they could. Cardiac atherosclerosis is generally restricted to hypothyroid or diabetic dogs. But there is a form of acute cardiac death due to arteriosclerosis to which dogs are susceptible.[1,2,4] It is associated with loss of myocytes in tunica media and deposition of amorphous material that may contain amyloid. Similar vascular changes occur in canine arteries elsewhere, particularly in brain and spleen, although these tend to be more common in senescent dogs.[5] The basis for cardiac arterial hyalinosis is unknown. There is no published evidence it is genetic or breed related, or that it is nutritionally induced. A report of ischemic heart disease in dogs in Sweden found that 14 of 16 dogs that died suddenly had hyaline arteriosclerosis.[3] Of these, 7 had acute and chronic infarcts, 6 had chronic infarcts alone, and 1 had no infarcts. It is interesting the dam of this dog died acutely and at a comparable age.

Owners are understandably upset when a dog dies unexpectedly and without premonitory signs. Often the first thought is poison. In this instance, it was helpful the veterinarian submitted an entire heart unfixed for evaluation. In sudden death, useful samples to submit are a full range of major organs, including brain, and an intact, unfixed heart that can be dissected at the laboratory. If poisoning needs to be excluded, good additional samples to submit are unfixed samples of liver, kidney, urine, stomach contents and a cerebral hemisphere.

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Donal O'Toole, WSVL Pathologist

## Seasonal Paralysis of Outdoor Pet Rabbits in Wyoming and Colorado

Many years around this time we see paralysis in adult domestic rabbits kept outdoors. Typically it occurs July – September. Rabbits develop posterior paralysis, and – at least the ones we receive – response to treatment is poor. Typically they are bright and alert, eat well, and have paresis/paralysis of pelvic limbs. A similar syndrome is seen in Colorado. They have seen

cases of again this year. We've seen cases from Cheyenne and Sheridan. When people have both indoor and outdoor rabbits, it affects only the outdoor ones – suggesting it is not directly transmissible rabbit-to-rabbit.

When we examine these animals we typically find multifocal areas of axonal degeneration or malacia with non-suppurative inflammation in spinal cord. Some lesions are suggestive of small migration tracts. No infectious agents have been isolated or seen in tissue section. A similar syndrome has been seen by pathologists in Washington State. In one instance they demonstrated a *Baylisascaris* sp. larva in a paralyzed rabbit's spinal cord. Although *Baylisascaris procyonis* (raccoon roundworm) is a well-documented cause of CNS disease in rabbits, it is difficult to credit the syndrome we see to *Baylisascaris* sp. These are large parasites about the size of a No. 11 bus, yet malacic lesions are small. Moreover, we should occasionally identify sections of the parasite in rabbits if they are a consistent feature.[3,4] If you have experience with this syndrome, and/or have cases of terminally affected rabbits, I would be interested in hearing from you. It seems possible lesions are due to a small migrating parasite that has found itself in the wrong host, such as *Cuterebra lepusculi*.[1] Roundworms other than *B. procyonis* are a consideration.[2] Anecdotal information you have of paralysis in wildlife that affected domestic rabbits have contact with, especially free-ranging rabbits or rodents, would be helpful.


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Donal O'Toole, WSVL Pathologist

## Laboratory Diagnosis of Bovine Trichomoniasis with LAMP

Bovine trichomoniasis is a venereal infection caused by *Tritrichomonas foetus*. Infection may lead to infertility, abortion and uterine infections in female cattle resulting in significant economic losses for producers. Bulls on the other hand are often asymptomatic carriers and are sources of infection in herds where natural service is used. Bovine trichomoniasis is widespread worldwide and has been found in many US states including Wyoming. Interestingly the same protozoan has been recognized as a cause of chronic diarrhea in the domesticated cat. It is also a common resident in the nasal passages and the digestive tract of pigs. At present, there is no reason to believe, and no data to support, that prevalence of bovine trichomoniasis is epidemiologically linked to infections in cats and pigs.

Main methods currently used in diagnosing bovine trichomoniasis are cell culture and polymerase chain reaction (PCR). In the former, samples are inoculated into Diamond's medium or InPouch™ TF. Cultures are checked daily for live parasites during the course of several days. Pros include low cost and easy to perform. Cons are ability to detect only live trichomonads, relatively low sensitivity of a single test, and false positives. PCR relies on amplification of protozoal DNA, is very sensitive and specific, and can be used to detect both live and dead trichomonads. Disadvantages include high cost and requirement of sophisticated and costly equipment such as a thermal cycler.

Another way to amplify DNA besides PCR is loop-mediated isothermal amplification (LAMP). LAMP has several advantages over PCR. It is many times more sensitive than PCR, and more tolerant to inhibitors in the sample such as urine and blood. Amplification can be done as fast as half an hour using a heat block. Test results can


be determined with the naked eye. In addition, reagents can be readily made and be commercialized for the end users. Samples may be directly added into the reagents without DNA extraction. DNA extraction is a bottle neck to move the test from diagnostic labs to the in-house facility of veterinarian's clinics. Here at the Parasitology Lab of WSVL we are developing a LAMP test for diagnosing bovine trichomoniasis.

At WSVL, the parasitology laboratory is exploring the use of LAMP in the diagnosis of trichomoniasis. We have amplified and sequenced a fragment of DNA from 12 strains of *T. foetus* collected from infected herds in Wyoming and South Dakota. The sequences of these 12 strains are 100% identical. Based on the DNA sequences three sets of primers were designed.

Our results showed that this LAMP reaction has sensitivity similar to PCR with 35 cycles. It is anticipated that LAMP reactions with other primer sets will be more sensitive than PCR or will shorten the reaction to half an hour, which is currently under investigation. Once the optimal set of primers is determined we will do final tune-up of reaction conditions. Afterwards, we will use LAMP on clinical samples submitted to WSVL, and determine its sensitivity and specificity using PCR as a "gold standard".

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Chaoqun Yao, WSVL
Parasitologist

**Equine Herpesvirus-1 Outbreak Related to a Cutting Horse Competition**

An outbreak of the neuropathogenic form of equine herpesvirus type 1 (EHV-1), termed equine herpesvirus myeloencephalopathy (EHM), originated at a cutting horse event in Utah held in late April to early May of 2011. With the first diagnosis, the National Cutting Horse Association notified the attendees and the State Veterinarians of the risk of horses having been infected with EHV-1, and exposing other horses upon returning to their home farms.

EHV-1 can cause disease in horses resulting in respiratory signs, abortions in mares, neonatal foal death, or neurological signs. EHV-1 is not a new disease and is ubiquitous in most horse populations. Many infections are subclinical and infection results in lifelong latency in the horse. It is likely that stress factors such as transport, strenuous physical exercise, fatigue or a suppressed immune system reactivate the virus resulting in clinical disease and renewed virus shedding. EHV-1 is most commonly spread by direct horse-to-horse contact, but can also be spread by contaminated hands, equipment and tack, and by short distance aerosol droplets. Clinical signs develop within 2-8 days following exposure. Neurological signs include ataxia, posterior incoordination and weakness, inability to rise, circling, head pressing, head tilt, and leaking urine.

Recommendation from the USDA and the American Association of Equine Practitioners included the following: 1) isolate all horses returning from the Utah event and monitor for signs of illness for at least 7 days; 2) maintain good sanitation practices to prevent the spread of infection (biosecurity); 3) test any horse with a fever, neurologic or respiratory signs by collecting

a blood sample and nasal swab for laboratory testing by PCR or virus isolation and serology; 4) quarantine any premise with a suspect or confirmed EHM for 21-28 days. A suspect case may not have detectable virus by PCR or virus isolation due to the transient nature of the virus in the blood or nasal secretions. An inexpensive paired sera assay using serum samples collected 3 weeks apart can often give us the diagnosis since a fourfold increase in antibody levels provides indirect evidence of recent infection. The final USDA report of the outbreak was released June 23, 2011. There were 90 confirmed cases of EHV-1 throughout the US related to the Utah cutting horse event. 54 of these cases were horses attending the event, and 36 cases were secondary, meaning horses acquiring the infection from competitors returning to their home farms. The state of Wyoming had one confirmed EHV-1 case directly related to the Utah event, but fortunately this horse had only a transient fever.

The quick response by the National Cutting Horse Association, State Veterinarians, and horse owners is likely to have greatly limited the extent of this outbreak. This outbreak is a reminder of the importance of good biosecurity and the value of separating new or returning horses for their first 7 days back on the farm or facility. There are vaccines labeled for protection of horses against the respiratory or abortigenic form of EHV-1, however, none of these vaccines have been shown to protect against the neurological form of the disease.

Myrna Miller, WSVL Virologist

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## FROM THE WYOMING DEPARTMENT OF HEALTH

The Wyoming Department of Health (WDH) is reporting a sharp increase statewide in potentially dangerous human Campylobacter bacterial infections this summer.

In June and July of 2011, WDH identified 38 cases of Campylobacter infections in Wyoming residents. The 10 year average for cases during June and July is 17. At least six people have been hospitalized. The reason for the increase in cases is not known.

Of the 38 cases reported in June and July, 19 had an exposure history where animal contact was the likely exposure. Exposure to cattle or dogs was reported most often.

Campylobacter infection is a common cause of bacterial diarrhea in the United States. Infected persons typically develop diarrhea (sometimes bloody), nausea, vomiting, stomach cramping, abdominal pain and fever for about one week.

Recommended precautions for avoiding Campylobacter infections include hand washing, avoiding consumption of unpasteurized milk or products made from unpasteurized milk, and wearing gloves when handling animals. Ill individuals should avoid work such as food preparation where they could pass along the infection to others.

Karl Musgrave
Public Health Veterinarian
Wyoming Department of Health