

Colorado State University Veterinary Diagnostic Laboratories



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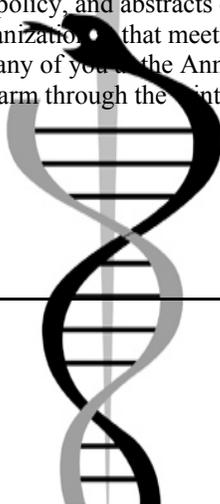
Fall/Winter 2006

Letter from the Director

Welcome to the Fall/Winter edition of LabLines! We have already had snow in Fort Collins and it seems as though winter is here. As always, there are many new things happening in the Diagnostic Laboratory. Inside are numerous articles which we hope you will find of interest. Also, note the new faculty, staff and students who have joined our laboratory in the recent months. Our 05/06 fiscal year ended June 30 and it is evident that our accessions and caseloads continue to grow. Our work with the National Animal Health Laboratory Network (NAHLN), a partnership between the American Association of Veterinary Laboratory Diagnosticians and the United States Department of Agriculture requires more testing for emerging and foreign animal diseases. Bovine spongiform encephalopathy testing has switched from the enhanced surveillance to the maintenance program; while testing for avian influenza, especially with the Department of Interior and USDA wildlife services through the NAHLN system continues to grow. The Colorado Avian Influenza Surveillance and Education Task Force coordinated by Dr. Kristy Pabilonia in the Diagnostic Laboratory is a multi-state agency collaborative effort to monitor for avian influenza in both the state and region.

We are excited to welcome Dr. Lora Ballweber to our parasitology section. She has introduced new diagnostic test procedures for parasitic disease and brings great experience and enthusiasm to our laboratory. We are in the process of hiring two new pathologists; one to fill the position vacated by Dr. Norrdin, who recently retired, and a new position to handle the increased pathology caseload through the Diagnostic Laboratory. In our Spring issue, we hope to be able to introduce these new pathologists.

It was wonderful to see many of you at the Colorado Veterinary Medical Association (CVMA) Annual Convention in Keystone, CO. Congratulations to Dr. Hana Van Campen who won the Faculty of the Year Award from the CVMA. It was also great to participate in the CVMA's 100 Year celebration. Later in October, at the American Association of Veterinary Laboratory Diagnosticians meeting in Minneapolis, MN, we had a great meeting. It was full of scientific information and policy, and abstracts of some of our presentations are in this issue. I was honored to become President of that organization at that meeting. The position offers a great many challenges for the next year. I look forward to seeing many of you at the Annual Conference at CSU or the CVMA's Winter Leadership Conference. Hope you all stay warm through the winter.



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A WALK ON THE WILD SIDE

—Colleen Duncan

In recent years, the role of wildlife in the epidemiology of zoonotic and emerging pathogens has been under increased scrutiny. Globally, wild animals are reservoirs for infectious diseases of substantial economic and public health significance. The epidemiology of infectious disease in wild animals has historically been under-researched due to difficulty in conducting representative studies, great expense and relative lack of motivation. Surveillance for disease in wild populations is complicated by similar limitations. However, it has become clear that early detection of morbidity and mortality events in all species, including wild animals, is critical in the identification of an emerging disease or infectious agent introduction.

As a post-doctoral fellow at Colorado State University, I am exploring the role of wildlife in emerging infectious diseases. One objective is to evaluate the capacity of the current infrastructure in the Rocky Mountain region to detect emerging disease patterns in wild populations. We are conducting a survey of all groups and agencies in a position to detect such health events in an attempt to identify the relative contribution of different groups to this surveillance effort. Finally, using information gleaned from the survey and from the evaluation of more structured wildlife health surveillance networks in North America, we will make recommendations for enhancing surveillance efforts and information sharing such that changes in disease patterns are readily detected and, where necessary, management strategies rapidly implemented.

The role of private practitioners in the field of emerging infectious disease often is classed as 'front-line' and thus, fundamental in the detection of new disease trends. Even if you do not work specifically with wild animals, we would appreciate your input. A short (~5min), on-line survey for veterinary clinics is available at:

<http://survey.cvmb.colostate.edu/wsb.dll/cgd132/VetSurvey.htm>. The survey can be completed by any individual, or multiple people, at the clinic who can comment on the clinic's role in wildlife disease surveillance. If you do not have Internet service and need a paper survey, or you have questions, please do not hesitate to contact me by E-mail (colleen.duncan@colostate.edu) or telephone (970-214-1779).

NEW EQUIPMENT IN THE CHEMISTRY/TOXICOLOGY LABORATORY

—Dwayne Hamar and Cathy Bedwell

This past summer the chemistry/toxicology section of the laboratory purchased a major piece of equipment – a liquid chromatograph with mass spectrometer as the detector (LC/MS). Neither of us have used a MS before so the learning curve will be steep.

The analysis begins by extracting compounds of interest from biological samples, injecting the extract onto a chromatographic column and eluting the separated compounds with a high pressure liquid solvent. The different compounds are eluted from the column at different times and go into the MS. The MS has the ability to determine the mass (molecular weight) of the compound and measure the amount of the compound present. The target molecular weight is then "trapped" in the MS, and this compound may be broken into molecular fragments that are characteristic of the original compound. The masses of the fragments then are determined. These breakdown compounds can be classified as a fingerprint of the original compound and referred to as MS/MS. This means we will have elution time, the mass of the original compound and the mass of the breakdown compounds within which to confirm the identity of the original compound.

LC/MS is a relatively new technique compared to GC/MS so method development will be a major undertaking. For GC/MS, a computer-

ized library of a large number of compounds exists. However, there is no pre-packaged library available for LC/MS. The LC/MS and LC/MS/MS parameters for each compound of interest need to be determined in our laboratory, requiring time and standards for every compound we want to be able to identify. Some of these standards are difficult to find and most are very expensive.



We have started with method development for anticoagulant rodenticides. We are using a relative short list of standards, ones that are common in Colorado. We hope to offer the anticoagulant rodenticide screen to our clients by the end of 2006. Cost and availability will be announced in LabLines, so stay tuned!

EQUINE VIRAL ARTERITIS IN 2006

--Hana Van Campen and Keith Roehr

Rumors of an equine viral arteritis (EVA) “outbreak” in racing quarter horses abounded this past summer. This may have been a surprise to many veterinarians when their clients were sent scrambling to have EVA serology tests performed in order for their horses to be transported across state lines. Many organizations require EVA tests of prospective sales horses prior to shipment.

Equine viral arteritis virus belongs to a unique virus family, the Arteriviridae, whose only other known members are porcine reproductive and respiratory syndrome (PRRS) virus, lactate dehydrogenase elevating virus and simian hemorrhagic fever virus. All of the members of

this family have a tropism for macrophages and endothelial cells which explains their transmission and the pathogenesis of the diseases.

In horses, the diseases caused by EVA virus have been recognized for more than 100 years. EVA infections result in respiratory disease, vasculitis with dependent edema, abortion storms and pneumonia in foals. The initial infection is via the respiratory tract where alveolar macrophages pick up the virus and carry it throughout the blood and tissues of the infected horse. Infection of the endothelium results in the vasculitis and damage to the blood vessels leading to edema, congestion and hemorrhages. In severe cases, the vascular damage leads to infarction and necrosis in multiple organs. The virus crosses the placenta and infects the fetuses of pregnant mares causing similar lesions. The death of the fetus ends in abortion.

In the intact colt or stallion, the virus is carried to the seminiferous tubules with only transient effects on fertility. More insidiously, the stallion may become a carrier of EVA virus and shed infectious virus in semen. Breeding or artificial insemination of susceptible mares with the semen from carrier stallions leads to infection in the mare. While the mare may be inapparently affected, she will shed infectious EVA virus from her respiratory tract and infect other susceptible horses. This scenario has led to multiple EVA abortions on individual farms.

To determine whether horses have been infected with EVA virus, serum is tested for neutralizing antibodies. Horses carry EVA SN titers for life, so only a four-fold or greater increase in titer between acute and convalescent samples can be used to identify a recent infection. EVA titers in mares, fillies and geldings simply indicate past **exposure or** infection. However, seropositive stallions require PCR testing or virus isolation to determine whether EVA virus is present in their semen, i.e., whether they are carriers and a potential source of infection. Approximately 50% of stallions that are infected will spontaneously cease shedding EAV in time. There are regulatory

methods that can be used to reclassify stallions as non-EAV shedders.

In some states, EVA prevention programs begin with serology tests on stallions prior to the breeding season. Seronegative (uninfected) stallions are then vaccinated and their vaccination history registered with the state. Annual vaccination of these stallions is required for registry.

In 2005, semen from two EVA-infected quarter horse stallions were shipped to a number of veterinary clinics and reproductive centers in the US. Mares bred to these stallions were shipped home as well, including premises in Colorado. Several farms **in other states** experienced clinical disease including respiratory infections and abortions. Investigation or “trace backs” of bred mares were performed by Colorado’s state veterinarians. Luckily, the horses tested did not have SN titer to EVA.

Please review the new proposed rule for control of EVA in Colorado at http://www.dora.state.co.us/pls/real/SB121_Web.Show_Rule?p_rule_id=2070.

EVA SN test = \$11.00, submit serum in a RTT; EVA PCR = \$30.00, VI = \$40.00, submit fresh or frozen semen.

***TRITRICHOMONAS FOETUS* IN THE CAT: GUIDELINES FOR FECAL COLLECTION AND SUBMISSION**

—Lora Ballweber

Tritrichomonas foetus is a flagellated protozoan that has been found to be associated with waxing/waning large bowel diarrhea in cats. The trophozoites, which may be present in a direct saline smear, are similar in appearance to those of *Giardia*, and, consequently, may be confused. Because the organism that infects the cat is the same organism associated with venereal disease in cattle, we can use the same diagnostic techniques – culture and PCR. However, the trophozoite, which is the only

stage that is passed, is quite fragile and the fecal sample must be handled correctly in order to optimize our chances of detecting the organism. Following are two methods that can be used for the collection and submission of feline fecal samples for the culture detection of *T. foetus*.

METHOD 1

1. Fecal must be freshly voided or collected with a fecal loop. Diarrheic feces are preferred.
2. DO NOT refrigerate the sample.
3. Trophozoite survival can be enhanced by:
 - a. removal of as much litter as possible
 - litter promotes dessication of feces which promotes the death of the organisms
 - b. mixing 2 parts fecal to 3 parts sterile, normal saline
 - saline helps maintain fecal moisture which promotes the survival of the organisms
 - remember, the more dilute the fecal sample is, the less likely the organism will be detected by culture
4. Ship overnight in an insulated container to protect from extreme heat or cold. Do not ship on ice. Be sure to request trichomonas fecal culture.

METHOD 2

1. Inoculate approximately 0.05 g freshly voided or loop-collected feces (approximately the size of a peppercorn) directly into the In-Pouch TF culture system.
2. Seal pouch, store upright and ship overnight in an insulated container to protect from extreme heat or cold. Do not ship on ice.

VERIFICATION - For an additional charge, PCR can be used to confirm the identity of *T. foetus* in culture-positive samples.

PLEASE NOTE: These recommendations apply only to samples submitted for the culture detection of trichomonad organisms. If additional diagnostic tests are required (eg, fecal exam), a separate sample must be collected and shipped on ice.

Trichomonas culture for cats—Submit as described above. Fee=\$15. Trichomonas PCR for cats—Submit as described above. Fee=\$35.

UTILITY OF RECTAL MUCOSAL BIOPSIES FOR DETECTING CHRONIC WASTING DISEASE IN ROCKY MOUNTAIN ELK (*Cervus elaphus nelsoni*)

--T.R. Spraker, T. Gidlewski, A. Balachandran, K.C. VerCauteren, L. Creekmore, and R.D. Munger

*Presented at the 49th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians.

Preclinical diagnostic tests for transmissible spongiform encephalopathies have been described for mule deer (*Odocoileus hemionus*), using biopsy tissues of palatine tonsil; for sheep, using lymphoid tissues from palatine tonsil, third eyelid and rectal mucosa. The utility of rectal mucosal biopsies was investigated in Rocky Mountain elk (*Cervus elaphus nelsoni*), a species for which there is not a live-animal diagnostic test. Postmortem rectal mucosal sections (PRMS) were examined from 308 elk from two herds that were depopulated. The results of the PRMS were compared to immunohistochemical (IHC) staining of the brain stem (vagus nucleus), retropharyngeal lymph nodes and palatine tonsil. Seven elk were found positive in the brain stem and cranial lymphoid tissues, however, only six of these elk were positive in the PRMS. The remaining 301 elk in which PrP^{CWD} was not detected in the brain stem and cranial lymphoid tissues also were free of PrP^{CWD} in the PRMS. Rectal mucosal biopsies (RMB) were taken from 421 live elk from three ranches in which at least two previous cases of CWD had occurred. Three positive non-clinical elk were found in one farm. These elk were killed and

CWD was confirmed. PrP^{CWD} also was found around neurons and in non-myelinated nerves within the submucosa. PrP^{CWD} also was found in the connective tissue of the lamina propria of the infected elk. Number of follicles counted in PRMS and biopsies decreased with age of animal. This procedure is easy and rapid to perform and may be suitable as a diagnostic test as part of an integrated management strategy to detect CWD in elk.

UPDATE ON THE DIAGNOSIS OF EHRLICHIA, NEORICKETTSIA, AND ANAPLASMA INFECTIONS OF DOGS AND CATS

--Michael R. Lappin

Based on genetic studies, the *Ehrlichia* group has been reorganized; some of the agents remained as *Ehrlichia* species and others were reclassified as *Anaplasma* species or *Neorickettsia* species. A number of the agents are associated with clinical disease in dogs or cats; these syndromes are collectively referred to as ehrlichiosis. *Ehrlichia canis* is still the most common agent and has been associated with illness in both dogs and cats. Granulocytic polyarthritis is associated with *E. ewingii* infection of some dogs in the central United States. DNA of *Anaplasma phagocytophilum* (previously *E. equi*, human granulocytic ehrlichial agent, *E. phagocytophila*, and granulocytic ehrlichial agent of dogs) now has been amplified from blood of dogs and cats with fever and thrombocytopenia and it also has been proven as a cause of polyarthritis in dogs. *Anaplasma platys* (previously *E. platys*) is occasionally associated with fever, thrombocytopenia and uveitis in dogs. *Neorickettia risticii* (previously *E. risticii*) is known as atypical ehrlichiosis; this agent has been detected in dogs with clinical syndromes identical to those associated with *E. canis*.

Clinical and laboratory manifestations of disease from infection with one or more of these agents can vary, but in general they

should be suspected in dogs or cats with fever, cytopenias (in particular anemia and thrombocytopenia), hyperglobulinemia, proteinuria, and polyarthritis. Occasionally, morulae of the organisms will be detected in blood or joint fluid. However, in most cases cytology is negative. The diagnosis usually is based on the demonstration of antibodies in serum or DNA of the organisms in blood or other fluids.

The point-of-care *E. canis* antibody assay (SNAP® 3DX, IDEXX Laboratories, Portland Maine) is a very good test for screening dogs with chronic ehrlichiosis as most of these dogs will be positive. However, some dogs with acute *E. canis* infection will be falsely negative in this test because of a relatively high cutoff value for a positive test. The IFA test we use has a lower positive cutoff than the point-of-care test and should be used with cases suspected to have acute *E. canis* infection.

While *E. canis* serological tests are very good for screening dogs for antibodies against *E. canis*, there are a number of potential problems. The most significant problem is the failure for *E. canis* assays to detect antibodies against *A. phagocytophilum*, *A. platys*, and *N. risticii* because of antigenic differences between the organisms. There are two ways to confirm ehrlichiosis from *A. phagocytophilum*, *A. platys*, and *N. risticii* infections. First, all dogs or cats with suspected ehrlichiosis can be serologically screened with assays for antibodies against *E. canis*, *A. phagocytophilum*, *A. platys*, and *N. risticii*. We offer a polymerase chain reaction assay (PCR) for use with blood and other fluids from dogs and cats with suspected ehrlichiosis. This assay has been titrated to amplify DNA from all known *Ehrlichia* species, *Anaplasma* species, and *Neorickettsia* species in the same PCR reaction and is an economical way to screen for multiple infections. The PCR assay also has the benefit of detecting very acute infections prior to the development of positive antibody titers. If a positive result is obtained, genetic sequencing can be performed to

determine the infecting species. After treatment, PCR assay results also can be used to gauge response to therapy.

In summary, the current recommendation for the screening of dogs or cats with clinical syndromes consistent with acute ehrlichiosis includes the combination of *E. canis* serology and *Ehrlichia/Anaplasma/Neorickettsia* PCR assay. It is optimal to collect samples for PCR assay assessment prior to starting antibiotic therapy to lessen the likelihood of false negative results. Visit the Specialized Infectious Disease website for more information.

<http://www.dlab.colostate.edu/images/20051031SpecializedUserGuide.pdf>

IFA for *Ehrlichia*—Submit 1ml of serum. Fee=\$30. PCR for *Ehrlichiosis*—Submit .05ml of whole blood, joint fluid, aqueous or CSF in a purple top tube. Fee=\$45.

EQUINE INFECTIOUS ANEMIA CASES FOUND IN COLORADO

—Christie Mayo and John Maulsby

A case of equine infectious anemia recently was diagnosed in Colorado and has sparked interest in the equine community. The case involved a mule that had a routine Coggin's performed (a Coggin's is an Agar Gel Immunodiffusion Assay). This serologic test revealed a positive result. The State Veterinarian's Office was notified and immediately sent veterinarians to investigate the situation. At that time, the farm was placed under quarantine until confirmatory testing was completed. Blood was collected from each horse on the entire farm and all other horses tested negative. The mule was euthanized and the farm was placed under quarantine for 60 days until repeat tests confirmed that all horses on the premises were, in fact, negative.

The virus that causes equine infectious anemia (EIA) disease is in the retroviridae family and specifically classified as a *lentivirus*. The prefix, *lent*, stems from the Latin *lentus* which

means slow. This is a progressive, life-long infection that can result in long incubation periods, persistent viremia or recurring febrile episodes and anemia. During episodes of latency, clinical signs can recur secondary to stress. Primary infection involves a 1-to-3 week incubation period followed by either the acute or subacute form of disease. Many animals will have a subacute form of the disease which often goes undetected because fevers are followed by recovery. However, the acute form can cause fever, anemia, jaundice, petechial hemorrhages of the mucosae, and result in death. The distribution of this disease is worldwide but is particularly noticed in the southeastern region of the United States where the environment is hot and humid and facilitates birth of flies of the *Tabanid* species.

Tabanid flies and *Culicoides*, both biting and blood-feeding insects, are suspected to be vectors that play a role in transmission of EIA. The feeding process of these insects is very different. A *Tabanid* fly actually slashes the skin when consuming a blood meal and is thought to be the primary vector in mechanical transmission. Rapid spread can occur when there is interruption of a fly's blood meal on a host harboring EIA, and then the fly continues its meal on another naïve host. It has been demonstrated that *Tabanid* flies are not capable of transmission four hours or more after feeding and do not travel more than 200 yards to feed on another host. This is a result of a mechanical rather than a biological vector in which transmission only occurs via infected mouthparts but does not live inside the vector. Other modes of transmission include colostrums, which can be passed from a mare, infecting the foal with virus or with antibodies against the virus. Most foals with EIA-infected mothers test positive 24 hours after birth. Those that have not contracted the virus will test negative three to six months after maternal antibodies have been cleared.

Two serologic tests, an ELISA and AGID, can aid in diagnosis of this disease. Serum should be submitted by an accredited veterinarian and submitted to an approved diagnostic laboratory.

The Coggin's (AGID) was developed in 1972 and has 95% accuracy detecting an EIA-virus infected horse. The AGID requires 24 hours before results can be reported. The ELISA test is much more sensitive and only requires four hours to perform. Neither test is useful to diagnose the acute form of EIA virus because acutely infected horses do not develop serum antibodies until 16 to 24 days after infection. Thus, in order to ensure acutely infected horses have had time to develop antibodies, a 60-day interval is recommended between initial and secondary serum sample.

Identification of infected horses is subject to the legal mandates of each state and it is important to comply with their laws. Reactors in Colorado should be permanently identified using the National Uniform tag number issued by the USDA. This number can be applied by an accredited veterinarian using a tattoo, brand or implant. The three approved procedures for handling infected equids are euthanasia, slaughter or quarantine in a screened area 200 yards from other horses for the remainder of life as is required in Colorado. While Colorado does not require horses to be identified by tattoo, brand or implant, it is important to review each state's requirements when shipping equids across state lines. If the horse must be moved to an approved research facility, it is important that the USDA provide a permit which acts as a legal document approving transportation. To aid in prevention and control, it is recommended to require EIA testing when a horse is purchased or transported to a new facility. The use of good fly control measures, hypodermis needle sterility ensuring separate needles for each equid, and disinfecting surgical instruments will help reduce spread of this life-threatening illness. While Colorado is not within the "hot zone," having only five horses test positive within the last two years, our state's veterinarians play an integral role in prevention and management of this disease.

EIA AGID—Submit 1ml serum in RTT. Fee=\$7.50.
EIA ELISA—Submit 1ml serum in RTT. Fee=\$12.

MORTALITY-ASSOCIATED LESIONS OF ENDURANCE SLED DOGS

**--M.M. Dennis, R.J. Basaraba,
D.A. Mosier, and G.H. Cantor**

*Presented at the 49th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians.

Little is known about causes of unexpected exercise-associated death in apparently healthy dogs, or the lesions associated with extreme physical exertion in animals. The objective of this study was to characterize the lesions and causes of fatalities in long-distance racing dogs that die while or soon after competing in the Iditarod Trail Sled Dog Race. This information is needed to provide insights into how to clinically recognize working dogs affected with exercise-associated, life-threatening conditions.

From 1995-2006, all dogs dying during or following competition while still in the care of the Iditarod Trail veterinary team, were examined by gross necropsy and histopathologic evaluation. Twenty-two dogs were evaluated during the study period, from a population of roughly 13,200 dogs that competed. In most cases, dogs were without premonitory clinical signs, collapsed while competing, and died immediately or while in carriage to the next race checkpoint. Lesions repeatedly observed in the study population included rhabdomyolysis (n=13), enteritis (n=10), gastritis (n=9), aspiration pneumonia (n=9), gastric ulceration (n=8), mild centrilobular hepatocellular necrosis (n=6) or hepatic fibrosis (n=3), gastric dilation (n=3), and mild cardiac myodegeneration and necrosis (n=3). Most deaths were attributed to aspiration of gastric contents (n=6), aspiration pneumonia (n=3), or acute blood loss (n=3) secondary to gastric ulceration. The cause of death was undetermined for seven dogs. All dogs with aspiration pneumonia had concurrent gastric mucosal lesions. No findings suggestive of neglect or abuse were found in any of the dogs.

Unexpected death is a rare event in conditioned sled dogs. Aspiration pneumonia, gastric mucosal lesions, and extensive myonecrosis were conditions recognized by this study that may result in death. Dogs that display clinical signs suggestive of any of these conditions should be released or excluded from strenuous exercise. The significance of hepatic lesions and enteritis is unknown since the prevalence of these lesions in the general population of racing dogs is unknown. Epidemiologic investigations are needed to clarify the risk for death certain factors pose endurance-racing dogs.

HOOKWORM ENTERITIS/BACTERIA COMPLEX AS A CAUSE OF MORTALITY IN CALIFORNIA SEA LION PUPS

**--D.S. Bolte, D.R. Hyatt, R.L. Delong,
T.R. Spraker, E.T. Lyons**

*Presented at the 49th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians.

As a follow-up to previous studies conducted in 2002, samples were collected from sea lion pups during the summer of 2005 from two islands, San Nicolas and San Miguel, off the coast of California. The objective of this study was to evaluate the cause of mortality in California sea lion pups as a result of hookworm infections. Freshly dead sea lion pups (60) were necropsied, and tissues from 47 of those animals which had gross lesions were sampled for culture (101 samples total). Small intestines were submitted on all 47 animals. Other tissues submitted for culture included peritoneal cavity (23), brain (8), heart (8), lung (5), joint (4), lymph node (3), kidney (2), and abscess (1). Small intestine samples were cultured aerobically and anaerobically, all other tissue samples were cultured for aerobes only. Traditional culture methods were used to detect and identify all bacteria present in each sample. Of the 47 animals sampled, all had a moderate to severe hookworm infestation. The most prevalent pathogenic organisms recovered were *Salmonella* sp. group B (10 animals), group C₂ (9 animals), group C₁ (6 animals), *Clostridium*

perfringens (14 animals), hemolytic *Escherichia coli* (37 animals), *Vibrio* sp. (4 animals), and *Klebsiella terrigena* (16 animals). Interestingly, some of the pathogens (such as *Salmonella* sp.) were found in samples from both islands, while others were found in samples from a single island (such as *Klebsiella terrigena*). This sampling was part of a multi-year study and the findings support the hypothesis that a hookworm enteritis/bacteremia complex is causing mortality in sea lion pups off the California coast.

UPDATE ON THE DIAGNOSIS OF FELINE BARTONELLOSIS

--Michael R. Lappin

Cats have been shown by culture or DNA amplification to be infected by *Bartonella henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana* and *B. bovis* (Brunt et al, 2006). Cats are the main reservoir hosts for *B. henselae* and *B. clarridgeiae* and are likely to be the reservoir for *B. koehlerae*. *Bartonella henselae* is the most common cause of cat scratch disease as well as bacillary angiomatosis and peliosis hepatis, clinical syndromes recognized most commonly in humans with AIDS. *Bartonella* species infect mainly endothelial cells and erythrocytes of cats. *Bartonella* species are then ingested by *Ctenocephalides felis* when the flea is taking the blood meal and is passed alive in flea feces. Infected flea feces are likely to contaminate cat claws or cat mouths during grooming and then *Bartonella* species are inoculated into humans or other cats when scratched or bitten. It also is possible that people are infected with *Bartonella* species when open wounds are contaminated with infected flea feces. Thus, one of the best strategies for avoiding *Bartonella*-associated illness in cats and people is to control fleas.

Bartonella species infection of cats is extremely common. In cats with fleas, up to 93% have tested positive for antibodies. In a recent study in the United States, we collected fleas from cats and attempted to amplify

Bartonella spp. DNA from flea digests as well as the blood of the cat. The prevalence rates for *B. henselae* in cats and their fleas were 34.8% and 22.8%, respectively. The prevalence rates for *B. clarridgeiae* in cats and their fleas were 20.7% and 19.6%, respectively. Most cats with serological evidence of exposure to a *Bartonella* spp., a *Bartonella* spp. cultured from blood, or microbial DNA amplified from blood by PCR assay are clinically normal. However, *Bartonella* spp. infection of cats also has been associated directly or indirectly with a variety of clinical manifestations like fever, uveitis, lethargy, lymphadenopathy, atypical seizures, and stomatitis/gingivitis.

Blood culture, PCR assay on blood, and serologic testing can be used to assess individual cats for *Bartonella* infection. While positive results in PCR assays or by blood culture prove current infection, serum antibodies only prove exposure to a *Bartonella* species. Culture is the gold standard for proving infection, but the organism is slow growing and so it may not be known that infection exists for up to 14 days. Thus, in clinically ill cats, PCR assay often is used to test for infection because the results return quickly. The PCR assay we use amplifies DNA of all *Bartonella* species known to infect cats and is performed weekly. While serologic testing can be used to determine whether an individual cat has been exposed, both seropositive and seronegative cats can be bacteremic, limiting the overall diagnostic utility of serologic testing alone. The assay we use is an ELISA that is known to detect IgG antibodies against *B. henselae* and *B. clarridgeiae*. Results of ELISA, IFA, and Western blot testing for *Bartonella* species antibodies are equivalent.

When assessing clinically ill cats for *Bartonella* species infection, the combination of serological testing and PCR assay (or culture) gives the most information. Cats negative in both assays are unlikely to be infected by a *Bartonella* species. In these cases, the diagnostic workup should be continued. The

organism should still be considered as a possible cause of the clinical syndrome if the cat is antibody positive, PCR assay negative, antibody negative, PCR assay positive, or positive in both assays. If there are no other more likely differentials, positive cats can be administered drugs with anti-*Bartonella* activity. The American Association of Feline Practitioners Panel Report recommends doxycycline at 10 mg/kg, PO, daily as the first drug of choice. Whether or not there is benefit to follow-up testing for antibodies or the organism in blood is currently unknown. However, because of potential zoonotic risk, flea control should be maintained in cats, year-round.

ELISA for *Bartonella*—Submit 1ml of serum. Fee=\$24.
PCR for *Bartonella* sp.—Submit 1ml blood in EDTA tube. Fee=\$45.

NEW TESTS IN PARASITOLOGY

Meriflour Crypto/Giardia IFA test – Detects both cryptosporidium and giardia in one test and can be used on any species of animal. The cost is \$20 and the test is setup one time each day. Ideal sample size is 3g of fresh feces. Samples must be in the Parasitology Laboratory by noon to receive results on the same day.

Feline Trichomonas culture and Feline Trichomonas PCR – To culture for the organisms, collect 1g of fresh feces. Remove as much of the cat litter from the sample as possible. Do not refrigerate the sample. Samples must be shipped overnight. The feces can be mixed with normal saline (2 parts feces to 3 parts normal saline) for transport. Fresh feces (approximately 0.05g, using a fecal loop) also can be inoculated directly into InPouch TF culture system and then transported to the Parasitology Laboratory. Fecal samples turned directly into the laboratory will be cultured in Diamonds media. Samples inoculated in Diamonds media are incubated and read in 24 hours with final results reported out after 72 hours of incubation. Samples submitted in the InPouch TF system must be incubated at room temperature. Final results may take up to two weeks after submission. The cost for the culture is \$15. PCR also is available to run on feces. The cost for Feline Trichomonas PCR is \$35.

MALIGNANT CATARRHAL FEVER ASSOCIATED WITH OVINE HERPESVIRUS-2 IN FREE-RANGING MULE DEER (*ODOCOILEUS HEMIONUS*) IN COLORADO

--P.C. Schultheiss, H. Van Campen,
T.R. Spraker, C. Bishop, L. Wolfe
and B. Podell

*Presented at the 49th Annual Meeting of the American Association Veterinary Laboratory Diagnosticians.

Malignant catarrhal fever (MCF) was diagnosed in four free-ranging mule deer (*Odocoileus hemionus*) in the winter of 2003. Diagnosis was based on typical histologic lesions of lymphocytic vasculitis in multiple organs and PCR identification of ovine herpesvirus-2 (OHV-2) viral genetic sequences in formalin-fixed tissues. The animals were from the Umcomphagre Plateau of southwestern Colorado. Deer were radio-collared in two separate winter range study areas as part of an ongoing mule deer study evaluating habitat effects on survival. Deer from these herds occasionally resided in close proximity to domestic sheep (*Ovis aries*), the reservoir host of OHV-2, in agricultural valleys adjacent to their winter range. MCF has been reported in many domestic and wildlife ruminant species but not confirmed in free-ranging mule deer. These cases indicate that fatal OHV-2 associated MCF can occur in free-ranging mule deer exposed to domestic sheep that overlap their range.

GET TO KNOW THE LABORATORY



Lora Ballweber—Born and raised in Cody, WY, Dr. Ballweber is an outdoor enthusiast with interests in photography and bow hunting. She is married to Jeff (they celebrated their 14th anniversary while moving to Fort Collins). She obtained her

Bachelor's degree in Animal Science in 1980 from the University of Wyoming; a Master of Science in Parasitology in 1982 from WY, a second Master's in Veterinary Medicine Science (Epidemiology, 1989) from Oregon State University, and a DVM in 1992 from Oregon State University through the WOI joint program. Dr. Ballweber is one of the few US veterinary parasitologists awarded Diplomate status in the European Veterinary Parasitology College. Diagnostic veterinary parasitology has always been among her duties in her various positions, the most recent prior to joining CSU at the CVM at Mississippi State University for 13 years. We welcome her and her expertise!

Joe Strecker joined us about one year ago as part of a College-wide push to rebuild existing IT systems using the new generation of technologies. He has worked with computers for many years. During graduate work at UCLA, he wrote programs to analyze electrical currents in cell membranes, leading to a PhD in Physiology in 1990. He continued his computing-intensive electrophysiology research as a post-doc with Dr. Ed Dudek's laboratory at CSU. Upon finding Fort Collins a terrific place to live and raise a family, Joe steered his career path from science to IT by obtaining an MS in Computer Information Systems at CSU in 1999. He was subsequently hired by the College of Veterinary Medicine and Biomedical Sciences Dean's Office as a programmer/analyst/server administrator. He was later appointed to manage the IT application development efforts for us, with the aim of providing computer programs to meet our business needs over the next 5-10 years. Joe is extremely impressed by the skill and dedication of the Diagnostic Laboratory staff he has met and is happy to be working with us to find ways that IT can make our group as effective as possible.



Christie Mayo joined us in mid-July. She received a BS at Clemson University and graduated from the University of Georgia last May with a DVM. Currently,

she has embarked on the journey of completing a combined microbiology residency and PhD program in infectious diseases. Colorado offers great opportunities for learning and exploring her interests of kayaking, biking and mountain climbing. Future goals include teaching and continuing research within a university setting.

!!CONSTRUCTION ZONE!! NEW FACILITIES

The Veterinary Diagnostic Laboratory is self-funding two major construction projects. In Fort Collins, we are building a new 4500 sq/ft office/sample entry building. This building will be located adjacent to our BSL3 and Histology laboratory buildings, across the parking lot north of the current lab (tan building with a green roof, south-facing entrance). In Rocky Ford, we are adding three additional laboratories and a conference room to the existing building. We also are replacing the entire leaky roof and upgrading the heating and air conditioning system.

Cost of the Fort Collins building is \$499,000 and the Rocky Ford Expansion is \$194,000. The Fort Collins building will be open in December and the Rocky Ford building should be completed early next year.



WHAT'S IN THIS ISSUE

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- New Tests in Parasitology
- Malignant Catarrhal Fever with Mule Deer
- New Facilities!



Knowledge to Go Places

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