Colorado State University Veterinary Diagnostic Laboratories

LAB LINES

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Letter from the Director

Spring has arrived in Colorado and with it, much needed moisture, though we hope for more! With the warm weather, we will see those seasonal diseases again. We have already had our first case of West Nile disease in a horse reported by the Rocky Ford Laboratory in early May. We had the first Colorado case of rabies in a bat for the year in April, associated with multiple human exposures. See inside for updates on new tests offered for a wide variety of diseases affecting many species. Highlights of this newsletter include information about BSE surveillance, our new BVD control program, and investigations of disease "outbreaks". We are also very pleased to be able to re-establish a dermatohistopathology-clinical consultation service with Dr. Bevier.

In January, we had our annual meeting with our external advisory committee (see inside for a listing of members). We had a very productive and useful meeting, and will use their suggestions to improve our services to you. We continue to work with the Colorado Department of Agriculture and the Colorado State Animal Response Team on emergency preparedness issues. We are also continuing our multi-agency work group to address carcass disposal issues in the state, for not only emergency response, but for day-to-day challenges. Along these lines, we are pleased to have a replacement incinerator on-site at our Grand Junction Laboratory, a collaborative effort between us and the Colorado Division of Wildlife. I was pleased to see many of you at both the January and May Colorado Veterinary Medical Association (CVMA) Leadership Conferences and look forward to seeing MANY of you in September at CVMA's Annual Conference in Steamboat Springs!

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EXTERNAL ADVISORY COMMITTEE

Every year in January at CSU's Annual Conference, we meet with our External Advisory Committee. We are grateful to these individuals who donate their time and give their expert advice to us at this meeting and throughout the year. This group helps direct and guide our future development. If you have any comments you would like to make, please contact them (or us directly!).

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USDA SELECTS COLORADO STATE UNIVERSITY VETERINARY DIAGNOSTIC LABORATORY AS PART OF NATIONAL NETWORK FOR HIGH VOLUME BSE TESTING

We have been selected to be a part of the US Department of Agriculture's national BSE laboratory network established to increase testing for bovine spongiform encephalopathy in the United States. We were chosen as one of only seven laboratories nationwide for high-throughput, or high volume, BSE testing.

Our high-security diagnostic laboratory was the only one of the seven facilities selected that was already equipped with the high-throughput equipment needed to conduct the necessary volume of testing. The equipment was installed in 2002 when we, in conjunction with the Colorado Division of Wildlife (CDOW), tested and validated the robotic system for use in the United States for chronic wasting disease (CWD) testing. The robotic system is part of the Bio-Rad rapid test, also validated by us and CDOW for CWD testing, and approved by the USDA for BSE testing.

The robotic system automates a portion of the testing procedure, speeding sample preparation and enabling laboratories to provide faster results using fewer technicians. With the automated system, we will be able to provide same-day results for BSE samples received by noon.

We have extensive experience using the Bio-Rad rapid test and high-throughput robotic equipment. Using this system, we have tested nearly 47,000 deer and elk samples for CWD in the last 18 months alone, and have a capacity of conducting 900 tests per day.

Robot

The seven selected national high-throughput laboratories have more than enough capacity to conduct USDA's targeted 268,000 BSE tests over the next 12 to 18 months. For example, we alone have the capacity to conduct more than 150,000 such tests per year.

In addition to our laboratory, the other six selected laboratories are—California Animal Health and Food Safety Lab Systems, University of California-Davis; Texas Veterinary Medical Diagnostic Laboratory-Collage Station; Wisconsin Animal health Laboratory-Madison; Washington State University Animal Disease Diagnostic Laboratory; Athens Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia; and NY State College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Cornell University. Other laboratories that meet specific criteria may be certified to analyze surveillance samples in the future.

We will be working closely with our USDA Area Veterinarian in Charge and our State Veterinarian to coordinate samples coming to us from Colorado and the surrounding states assigned to us. The testing program is to begin June 1.

Tritrichomonas foetus DIAGNOSTIC TESTING BY CULTURE AND CONFIRMATORY PCR

—Brendan Podell

Tritrichomonas foetus, the causative agent of bovine tritrichomonosis, is a sexually transmitted disease transferred from asymptomatic bulls to heifers or cows that may lead to infertility and abortion. A PCR test is now being offered for diagnosis of *Tritrichomonas foetus*. Diagnostic results on culture can be hampered by contamination of samples with intestinal trichomonad protozoa other than *Tritrichomonas foetus*. This PCR test differentiates *T. foetus* from all other trichomonads that may potentially contaminate a sample. PCR testing confirms both the presence of any trichomonad species, as well as *T. foetus* specifically. This PCR test is mandatory as a confirmatory test for diagnosis following a positive culture of any trichomonad organism. Send in appropriate samples of preputial wash fluid for in vitro cultivation. *Tritrichomonas* culture is available at all three laboratories—Fort Collins, Grand Junction, and Rocky Ford. PCR will confirm results if necessary.

Tritrichomonas foetus culture. Submit preputial wash in lactated ringers or in pouch. Fee=$6/1-50; $5/51+. PCR test. Submit as described above. Fee=$30.

INVESTIGATION OF A LARGE FOOD ANIMAL SUDDEN DEATH SYNDROME

—Darrel Schweitzer/Western Slope

Once again, a large die-off of a food animal species has caught the attention of the news media in western Colorado and throughout the state. These kinds of events have occurred sporadically for the 26 years I have been involved here. Prior to 9-11-01, they garnered little attention. All that has changed, so this case may help veterinary practitioners facing similar circumstances.

In this case, a producer lost 31 cows and younger stock during a period of two days, most of the animals on the first morning of the outbreak. The animals had not been observed as abnormal the prior evening, but were noted to be down and struggling in the morning. They had been fed a large round bale of hay the previous day, one of several taken from the same hayfield. They also were being fed rolled corn with molasses (which had been fed for the previous three weeks), and a supplement in the form of a lick tub. Water was via a ditch into a small stock tank with overflow into a pond. There were many old cars in the pasture. Another group of cattle on the premises were fed hay from a different source, no grain, but water from the same source. There were no losses in this group.

The following clinical signs in animals still alive were reported by the referring veterinarian: In a yearling bull – ataxia, course muscle tremors, poor control of pelvic limbs, normal pupils, pale membranes, and no diarrhea, but still able to move away when approached. In an adult cow – down, unable to rise, slight bloat, clear lung sounds, no rumen sounds, pale membranes, pupils normal, no diarrhea, and a slight serous discharge from nostrils.

There was no shortage of opinions as to what might be wrong. The media seemed to have their own sensational opinion, law enforcement another, the owner another, and still others. The referring veterinarian was under tremendous pressure to come up with an answer.

As veterinarians, you are well prepared to handle such cases. Don't be afraid to get involved, but remember you are the expert. Don't let anyone pressure you into making rash pronouncements. If this kind of case can be solved, it will be solved by rational, methodical procedures. It's good to establish at the outset that testing will take time. No laboratory can provide instantaneous results for complicated testing procedures. However, you will undoubtedly need the assistance of a diagnostic laboratory. Let's review what may be involved by looking at this case as an example.

You need to take a good history and supply it to the laboratory. The present case is testament to that fact because the history led to a diagnosis. To start, the history led us toward intoxication as a possible cause. While some infectious diseases may be as rapid acting, other clinical signs usually are associated. First of all, the lick tub. Because these typically contain urea as a protein source, urea toxicosis was a consideration. In drought conditions, such as we've been experiencing the past few years, nitrate can build up in plants, another consideration, both from the feed and the open ditch water source. Poisonous plants always should be considered. Included in the history was the fact that the owner knew some narrow-leafed milkweed grew in the hayfield. This plant often is involved in acute poisoning in this area, so it became a prime suspect, given the signs observed. The history mentioned old cars in the pasture. While the referring veterinarian looked specifically for old batteries and found none, lead poisoning was nevertheless a consideration to

explore. The history, likewise, ruled out some possibilities. Organophosphates, often involved in rapid deaths, were low on the list of possibilities because of information given.

Next, the laboratory needs the materials necessary to investigate these possibilities. In this case, we were provided with an excellent selection of materials to explore the possibilities. Without proper samples, the diagnosis would have been missed. At a minimum, in suspected toxicology cases you should obtain liver, kidney, fat, stomach (or rumen) contents, brain, blood (even if hemolyzed), ocular fluid (or an intact eyeball), and urine (if available). In order to help rule out infectious causes, lung, heart, spleen, lymph node, and gut also should be included. Samples should be submitted fresh, frozen (quickly), and in formalin. Environmental samples should include any feedstuffs, including supplements, and water from all sources. Of course, your necropsy findings will dictate any additional samples that might be appropriate. Remember, unneeded samples can always be discarded, but you usually cannot go back to get more. Again, be sure to include your necropsy observations. This can help the laboratory prioritize testing, as it did in this case.

In the above case, since we had all the necessary materials at hand, bacterial culture quickly ruled out a bacterial infection and histopathology ruled out any tissue abnormalities. Thus, any toxins that cause tissue damage also could be ruled out. Since the prime suspect, western whorled milkweed (*Asclepias subverticillata*), causes no observable tissue damage, it moved up on the priority list.

A. subverticillata (whorled milkweed)

The toxicology laboratory also was able to rule out nitrate, urea (ammonia), and lead, and several other considerations as being involved. It is possible to perform microanalysis of plants in rumen content and this was performed at the Texas Veterinary Medical Diagnostic Laboratory. *Asclepias subverticillata* was found in abundance in the rumen and the diagnosis was established.

What went right? Everything. Lack of any one of the materials submitted, but especially rumen content, would have been critical. Because of the excellent history and necropsy findings, we were able to intelligently select and prioritize testing, saving money on tests we otherwise might have felt necessary to initiate. Here are some other observations specific to this case. The remaining large round hay bale was examined for the presence of milkweed and none was found. This can be typical. Milkweed grows in relatively large stands because it spreads by horizontal underground roots. Often it is incorporated into only a portion of a large bale, or a few small bales. This portion is then completely consumed before any signs are noted, so lack of a plant in any remaining hay is not a reason to rule it out as a possibility. This plant, and several others, are quite toxic so plant material may not have time to be distributed throughout the rumen. For this reason, we prefer to sample rumen contents from the reticulum and anterior rumen.

RENEWED DERMATOHISTOPATHOLOGY SERVICE

--Diane Bevier

What do we offer?—We offer an enhanced report that takes the regular pathology report one step further to help you manage your tough skin cases. The report offers a more detailed description of findings, as well as differential diagnoses. The report also offers advice regarding further diagnostic steps and specific therapeutic information where appropriate. Dosages of medications are cited, in addition to common side effects. References to current literature appropriate to the case may be included. Occasional reports may state that it would be helpful to re-biopsy. *The Dermatohistopathology service is not intended to take the place of a case referral!*

How do you use this service?—You will need to complete a special dermatopathology request form. In dermatopathology, we frequently obtain results which are compatible or supportive of a specific disease rather than definitively diagnostic. The correlation of the pathologists' findings with the clinicians' clinical and historical findings are a critical factor in optimizing the results you get from this service. An increased fee is charged, as this service is more time-consuming than our regular pathology service.

Who runs the service?—The CSU Veterinary Diagnostic Laboratory is running the service in conjunction with Dr. Diane Bevier, DACVD, Purdue University. Dr. Bevier is the major contact. She is working with the ACVP Board Certified Pathologists who are currently doing biopsy service. These are Drs. Pat Schultheiss, Karamjeet Pandher, EJ Ehrhart, Gary Mason, Randy Basaraba, and Barb Powers. Dr. Diane Devier graduated from Michigan State University in 1976 and completed a dermatology residency at the University of Florida, becoming board certified in veterinary

dermatopathology in 1983. Dr. Bevier has worked in general small animal practice, private referral dermatology practice, industry, and as a university professor. She has been involved in the past in a mail-in biopsy and dermatology consultation service reviewing more than 1,500 cases. Dr. Bevier currently is a Clinical Associate Professor of Dermatology at Purdue University and gives numerous continuing education sessions each year. Dr. Diane Bevier comes with two decades of clinical dermatology, dermatopathology, and teaching experience to work with the CSU pathologists and continue the excellent dermatopathology service started at CSU with Dr. Sonya Bettenay in 1999.

Does this give your clients double the value?—We certainly believe it does! In fact, depending on the information you supply, it may be the most cost-effective test you can offer for your challenging dermatology cases!

Dermatology Service—Submit skin biopsies in 10% neutral buffered formalin (mailers available). Fee=$55/per report. This includes three glass slides (which can hold as many as 15 punch biopsies).

AN OVERVIEW OF COLORADO'S BVD CONTROL PROGRAM

—James Kennedy/Rocky Ford

Bovine viral diarrhea (BVD) decreases financial performance for the beef cow/calf producer. New technology in detecting BVD virus now will allow for the development of a control and eradication program from our state's beef herd. National organizations, such as AABP and AVC, are actively pursuing BVD control and eradication programs and, if Colorado is to maintain its position as a beef producer, it also must engage in a control and eradication program. BVD silently steals profits from the cow/calf producer by decreasing reproductive performance. The primary source of infection has been determined to be persistently infected animals, a result of exposure to the virus during gestation. The economic loss of the disease for the cow/calf producer was monitored over a 10-year period. Losses from the disease ranged from $14.85 to $24.84 per head in herds with known infection. Seed stock producers now are being asked to provide information as to the potential of PI (persistently infected) cattle as a part of their sale agreement. Although the disease in the cow herd quietly diminishes profits, if a persistently infected animal reaches the feedlot, the outcome can become a major financial impact to the cattle feeder. A single PI animal in a pen of cattle will infect as many as 30% of its pen mates and 20% of the cattle in neighboring pens. Feedlots are anxious to minimize the risk of PI animals but realize that the problem must be addressed at the cow/calf level. BVD poses no human risk but its ability to decrease livestock production and increase the expense of production demand that its economic impact be minimized through management and biosecurity. Participating in a BVD control and eradication program will improve livestock production for producers through improved waning rates, greater weaning weights, and by lowered morbidities and mortalities. Colorado State University Veterinary Diagnostic Laboratories back the BVD Program. The program is voluntary and its intent is to increase the marketability and performance of Colorado cattle. Since the primary source of BVD infection is persistently infected animals, this program will address methods directed at eliminating them from the cow herd, practicing good biosecurity, and implementation of effective vaccination programs.

BVD PI calves

The program is structured in three levels to allow producers to commit to the program at an expense that matches their production and financial goals. When each level is completed, the producer will be given a letter indicating the level of completion. Letters will be valid for one year, renewable by completing the outlined requirements of each level. Once a producer attains a certified BVD PI-free status, he will be able to maintain that status by testing 10% of his calf crop, or a maximum of 14 animals, at weaning using serological tests for Type I and Type II BVD antibodies. All titers must be below 1:512 to maintain the certified free status.

Level 1—Consists of simply completing a questionnaire. The questionnaire will be scored and that score will be used to assign a BVD risk from 1 to 3. Risk one would be assigned to herds evaluated as being a low risk herd with probability of infection being less than 10%, while risk 3 would be herds whose probability of infection is greater than 20%. When cattle are sold, the level and risk would be provided at the owner's discretion. Questionnaires will be scored by the owner's veterinarian and submitted to the CSU Veterinary Diagnostic Laboratory at Rocky Ford.

Level 2—Consists of meeting the requirements of Level 1 with a risk value of 1. Herds designated as Level 2 will be required to test all purchased and raised replacement breeding stock and their offspring, plus the current years calf crop using appropriate testing procedures which include ELISA, IHC, PCR, and pooled PCR testing. Additionally, a vaccination program including BVD must be implemented and will be validated through submission of receipt of purchase of appropriate BVD vaccines. The CSU Veterinary Diagnostic Laboratory at Rocky Ford will validate test records.

Level 3—Requires testing of any cattle not represented by the testing of the previous calf crop, such as bulls, cows without calves by their side, show cattle, etc. Any positive animals must be removed from the herd to be certified at Level 3. Testing to monitor the BVD-free status of the herd will be performed yearly through serological tests and the testing of replacement animals. The CSU Veterinary Diagnostic Laboratory at Rocky Ford will validate test records.

BVD IMMUNOHISTOCHEMISTRY AND CAPTURE ELISA OF EAR NOTCHES

—Hana Van Campen

We have added immunohistochemical staining of ear notches for BVD virus antigen (IHC-BVD) to its battery of BVDV tests. The IHC-BVD is one of the preferred tests for detecting persistently infected (PI) calves under four months of age, as maternal antibodies will not interfere with the detection of virus. Ear notches of approximately 1x1x1cm, made with a pig ear notcher, should be placed in 10% buffered formalin in individual, labeled tubes. Because the antigen is sensitive to denaturation by formalin, samples should be submitted within five days of collection. Alternatively, ear notch samples can be used for the c-ELISA test for faster results. Please call us if you are sending more than 50 ear notches in a single submission.

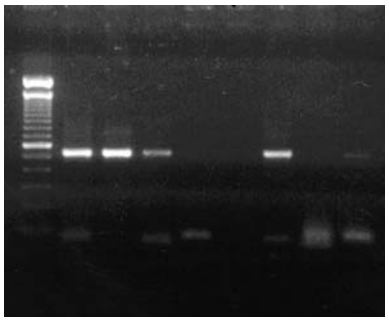
BVD IHC—Submit ear notches in 10% buffered formalin. Fee=$20/1-6 samples.
BVD cELISA—Submit ear notches in saline. Fee=$7/1-10; $5/11-50; $4/51+.

NEW PCR ASSAYS

—Dr. Michael Lappin/VTH

We are pleased to announce the addition of nine new PCR assays. We are now offering PCR assays for the amplication of *Toxoplasma gondii*, *Neospora caninum, Mycoplasma haemofelis* (previously *Haemobartonella felis* large form), *M. haemominutum* (previously *H. felis* small form), *Ehrlichia canis, Anaplasma phagocytophilum* (previously *Ehrlichia equi*), *Neorickettsia risticii* (previously *E. risticii*), *Bartonella henselae* (cat scratch disease agent), and *B. vinsonii*. The following are the indications, sample handling recommendations, costs, and interpretations that we will be using for this assay. For additional information contact Melissa Brewer or Jennifer Hawley at 970-297-0367 or Dr. Michael Lappin at mlappin@colostate.edu. The *Mycoplasmas/Ehrlichia/Ana-plasma/Neorickettsia/Bartonella* PCR assays are performed on Monday and Tuesday with reports sent on Wednesday. The *Toxoplasma/Neospora* PCR assays are performed on Wednesday and Thursday with reports sent on Friday.

Toxoplasma gondii—Use this PCR assay with blood, aqueous humor, CSF, BAL, or TTW samples, as well as tissue aspirates or biopsies from dogs or cats with suspected clinical toxoplasmosis. The assay results should be interpreted with those of serum IgG and IgM antibody assays. Place fluids and aspirates in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Formalin-fixed tissues also can be assayed, but the sensitivity is less than with fresh tissue. Detection of *T. gondii* DNA in fluids or tissue documents infection. Healthy cats have been shown to be transiently positive in blood, aqueous humor, and CSF, as the organism disseminates after primary exposure. Not all positive cats are clinically ill. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur, especially with formalin-fixed tissues.



PCR gel

Neospora caninum—Use this PCR with blood, aqueous humor, CSF, BAL, or TTW samples, as well as tissue aspirates or biopsies from dogs with suspected clinical neosporosis. The assay results should be interpreted with those of serum IgG antibody assay. Place fluids and aspirates in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Formalin-fixed tissues also can be assayed, but the sensitivity is less than with fresh tissue. Detection of *N. caninum* DNA in fluids or tissue documents infection. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur, especially with formalin-fixed tissues.

***Cryptosporidium* spp.—**Dogs and cats are infected by a number of *Cryptosporidium* spp. The organisms most commonly are associated with small bowel diarrhea. Since the organism is so small, routine fecal examination techniques can be falsely negative. This PCR assay is 10-100X more sensitive than other *Cryptosporidium* detection procedures and is indicated in the workup of dogs and cats with unexplained small bowel diarrhea. Ship feces to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Positive test results document infection by a *Cryptosporidium* spp. The amplicon can be sequenced to determine the infecting organism, if requested. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur.

***Mycoplasma haemofelis* and *M. haemominutum* (previously *Haemobartonella felis)*—**Use this PCR assay with blood or bone marrow of cats with hemolytic anemia or fever of unknown origin. It also is indicated for screening potential blood donor cats. Place blood or bone marrow in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Detection of DNA of either *M. haemofelis* or *M. haemominutum* documents infection. *M. haemofelis* is generally more pathogenic than *M. haemominutum* and so positive test results usually correlate to clinical illness. *M. haemominutum* DNA is detected in blood of about 10% of healthy cats and so positive test results do not always correlate with clinical illness. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur.

***Ehrlichia, Anaplasma,* and *Neorickettsia*--**Dogs and cats are known to be infected with *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Neorickettsia risticii*. This PCR sample amplifies DNA of each of these organisms as well as other sequenced *Ehrlichia* spp. of dogs. Use this PCR assay with blood, aqueous humor, CSF, or joint fluid from dogs or cats with fever, cytopenias, uveitis, polyarthritis, proteinuria, or hyperglobulinemia. The assay also is indicated for screening dog or cat blood donors. Pack fluids in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Positive test results document infection by an *Ehrlichia, Anaplasma*, or *Neorickettsia* spp. The amplicon can be sequenced to determine the infecting organism, if requested. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur. If negative, the assay results should be interpreted with those of serum antibody assays.

***Bartonella* spp.—**Dogs can be infected by *B. vinsonii* and *B. henselae*; cats can be infected with *B. henselae, B. clarridgeaie, B. weissi,* and *B. koelarae. B. vinsonii* infection of dogs is associated most frequently with myocarditis, vasculitis, thrombocytopenia, polyarthritis, and hemolytic anemia. *B. henselae* infection of cats most commonly is associated with fever, uveitis, gingivitis, and lymphadenopathy. This PCR assay amplifies DNA and differentiates each of these organisms as well as other sequenced *Bartonella* spp. of dogs. Use this PCR assay with blood, aqueous humor, CSF, or joint fluid from dogs or cats with fever, cytopenias, uveitis, or polyarthritis. The assay also is indicated for screening dog or cat blood donors. Place fluids in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Positive test results document infection by a *Bartonella* spp. Failure to detect organismal DNA generally suggests the organism is not the cause of the clinical syndrome. However, false negative results can occur. If negative, and there is still concern that *Bartonella* spp. are involved, the assay results should be interpreted with those of serum antibody assays.

Screening PCR assay panel for canine and feline blood donor—Healthy dogs and cats can be carriers of *Mycoplasma haemofelis* (cats; previously *Haemobartonella felis*), *M. haemominutum* (cats; previously *H. felis*), *M. haemocanis* (dogs; previously *H. canis*), *Bartonella* spp. (dogs and cats), *Ehrlichia* spp. (dogs and cats), *Anaplasma phagocytophilum* (dogs and cats; previously *E. equi* and granulocytic *Ehrlichia*), and *Neorickettsia risticii* (dogs and cats; previously *E. risticii)*. Each of these agents can be transmitted by blood transfusion and so testing is potentially indicated for blood donor dogs and cats. Place blood in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Detection of DNA of any of the infectious agents documents infection. Positive animals should not be used as blood donors. It is unknown whether treatment eliminates the carrier phase. Failure to detect organismal DNA generally suggests the animal is not infected. However, false negative results can occur.

Screening PCR assay panel for dogs and cats with fever of unknown origin—Fever has been associated with *Mycoplasma haemofelis* (cats), *M. haemominutum* (cats)*, M. haemocanis* (dogs*), Bartonella* spp. (dogs and cats), *Ehrlichia* spp. (dogs and cats), *Anaplasma phagocytophilum* (dogs and cats), and *Neorickettsia risticii* (dogs and cats*)*. Place blood in EDTA and ship to the laboratory with a cold pack. The samples can be frozen indefinitely prior to shipping. Detection of DNA of any of the infectious agents documents infection and indicates appropriate treatment if the animal is currently febrile. Failure to detect organismal DNA generally suggests the animal is not infected. However, false negative results can occur.

PCR testing—Submit samples as described above. PCR for *T. gondii, N. caninum, Cryptosporidia* sp. Fee=$35.00/sample. PCR for *M. haemofelis, M. haemominutum, Ehrlichia, Anaplasma* and *Neorichettsia*. Fee=$45.00/sample. (Combo *Mycoplasma, Ehrlichia, Anaplasma, Neorickettsia.* Fee=$60; Combo plus *Bartonella.* Fee=$105).

HEMANGIOSARCOMA IN SKIN AND OTHER NON-VISCERAL LOCATIONS

—Patricia Schultheiss

We have diagnosed numerous cases of hemangiosarcoma in skin and other non-visceral specimens. In contrast to visceral hemangiosarcomas, which are known to have a grave prognosis, little information was available about the non-visceral form. To gather information about these tumors, we reviewed cases from a six-year period and contacted 112 veterinarians who had submitted cases during a 12-month period to find out clinical outcome. A review of the cases revealed that visceral hemangiosarcomas represented less than 2% and non-visceral hemangiosarcoma less than 1% of canine specimens submitted for histologic examination. Most non-visceral hemangiosarcomas of dogs occurred in the skin. A wide variety of dog breeds were affected, but Italian

greyhounds, greyhounds and whippets were over-represented. Hemangio-sarcomas are less common in cats and usually occur in the skin. They are rare in other animal species as well. Animals with non-visceral hemangiosarcomas are usually mature; dogs and cats average 10 years of age. The tumors develop in many different locations and there is no sex predilection. Submitting veterinarians supplied information about the clinical outcome of 76 cases of non-visceral hemangiosarcomas, representing 66 dogs and 10 cats. Completeness of excision of the tumor is the most important factor that can be used in predicting clinical outcome for an affected animal. In all cases in which the animals were clinically normal for at least one year after surgical removal of a non-visceral hemangiosarcoma, the margins were free of neoplastic cells. Tumors which could not be removed completely and tumors in bone and muscle had a poor prognosis. This information about expected outcomes and the importance of complete excision is valuable for veterinarians. The cooperation of laboratory clients in this is greatly appreciated.

Histopathology diagnostics—Submit sample in formalin (mailers available from the laboratory). Fee=$30.00 for up to 3 slides.

ANIMAL CANCER CENTER ANNOUNCEMENT
Clinical Study for Canine Patients with Hemangiosarcoma

To-date, canine hemangiosarcoma is not a curable disease. We are investigating whether continuous administration of low dose chemotherapy designed to block angiogenesis will extend the disease free interval of canine patients with surgically resected splenic hemangiosarcoma.

Eligible patents are those that have been diagnosed with splenic hemangiosarcoma that has not metastasized to other organs. Patients may not have received previous chemotherapy treatment for hemangiosarcomas and certain medication/supplements are not allowed to be administered concurrently. Patients may not have any pre-existing diseases that would prevent them from completing the study. The owner is financially responsible for the initial diagnosis, surgical treatments, and staging tests. Thereafter, the study will cover all study-related treatments.

For further information, please consult: www.csuanimalcancercenter.org or contact the clinical study coordinator at 970-297-4001.

DIAGNOSTIC FEATURES OF EQUINE ENDOCRINE-METABOLIC DISORDERS

—Charlie Dickinson/VTH

The major equine endocrine-metabolic disorders of horses include pituitary pars intermedia dysfunction (PPID, equine Cushing's-like syndrome), equine metabolic syndrome (EMS, peripheral Cushing's-like syndrome, fat horses), and hyperlipemia/Hyperlipidemia Syndrome (HHS). A major clinicopathologic feature of these conditions is insulin resistance. Insulin insensitivity occurs as a consequence of hypercortisolism (PPID), obesity (EMS) and, in some individuals, constitutional predisposition. Hyperlipemia/lipidemia is an extreme manifestation of insulin resistance that occurs primarily in ponies, minis, donkeys and mules because they are inherently insulin insensitive relative to other equids. Both PPID and obesity seem to predispose to laminitis and is often the major clinical problem associated with these conditions. Animals with a predisposition to insulin resistance and obesity (some horses, most ponies, and donkeys) that also develop PPID, seem particularly prone to laminitis as well as the severe derangements in carbohydrate and lipid metabolism characteristic of HHS.

Pituitary pars intermedia dysfunction—A presumptive diagnosis of PPID often can be made based on signalment, history and clinical signs. PPID is generally a condition of aged horses, although some may develop the condition in the mid-to-late teens. The classic clinical sign of PPID is hirsutism (often accompanied by hyperhydrosis) and, when present, is virtually diagnostic. However, not all horses with PPID are clearly hirsute, particularly in early stages of the disease. Additional clinical signs can include muscle wasting (often interpreted as weight loss), generalized loss of condition, recurrent laminitis, polyuria/polydypsia, recurrent infections and infestations, and lethargy. Seizures and other neurological signs have been reported. Abnormal fat deposits such as "cresty neck" and supra-orbital fat deposition are sometimes noted. It is important to realize that obesity and abnormal fat distribution also are characteristic of and most consistent with equine metabolic syndrome. A clinical impression exists that obese, insulin-resistant horses are particularly prone to develop PPID as they age.

Hematological abnormalities observed in horses with PPID include neutrophilia, lymphopenia and anemia. In most individuals these changes are mild. When present, hyperglycemia is probably the most relevant clinical laboratory abnormality associated with PPID. This is because very few, if any, other conditions of horses are associated with moderate to severe persistent hyperglycemia at rest. Resting hyperglycemia is highly suggestive of PPID. Additional biochemical abnormalities reported include increased serum hepatic enzyme elevations, hypercholesterolemia and hyperlipidemia. In some affected horses these biochemical changes are minimal to absent. However, in advanced cases, especially when PPID develops in obese or otherwise insulin insensitive horses, significant hypertriglyceridemia can occur. This can lead to serious biochemical derangements as discussed below under hyperlipemia/hyperlipidemia.

The overnight dexamethasone suppression test (DST) is the most practical and reliable means of confirming PPID. In the late afternoon (approximately 5PM), the horse is given an intramuscular injection of dexamethasone at a dosage of 40μg/kg (20mg/500kg horse). Approximately 19 hours later (noon the next day), a serum sample is procured for cortisol measurement. In normal horses, serum cortisol will be

suppressed to a level below one μg/dL. Cortisol levels greater than 1μg/dL strongly support the diagnosis of PPID.

Elevations in plasma ACTH and insulin can be supportive of a diagnosis of PPID, but are probably most useful in evaluating response to treatment. An abnormal elevation in endogenous ACTH is highly suggestive of PPID, but a normal value does not rule out the disease. If ACTH levels are abnormally elevated, decreasing ACTH levels subsequent to initiation of treatment suggests a favorable response. Likewise, hyperinsulinemia may be present in PPID due to the glucocounter-regulatory effects of cortisol, yet normal insulin levels do not rule out PPID. In addition, hyperinsulinemia is characteristic of other conditions, most notably equine metabolic syndrome. Nonetheless, in PPID patients that are hyperinsulinemic, a sustained reduction in plasma insulin levels may be indicative of a positive response to treatment.

Obesity in Horses—In humans, metabolic syndrome refers to the insulin-refractory state associated with obesity. The essential metabolic dysfunction is insulin resistance, which develops in association with excess dietary carbohydrate intake, lack of exercise, and/or genetic predisposition. In horses, as in humans and other species, excess dietary concentrate and inactivity encourages the deposition of excess adipose tissue. Obesity itself exacerbates insulin insensitivity and, in this sense, metabolic syndrome is a self-perpetuating condition. Some breeds (ponies, donkeys, Morgan horses, Pasos, mustangs) and individuals appear to be constitutionally predisposed to obesity and deposit adipose tissue more readily under conditions of relative inactivity and dietary excess. These are the classic "easy keepers" and fat ponies. Humans (and dogs, cats, etc) with metabolic syndrome are at risk for type II diabetes mellitus (the pre-diabetic state). The major clinical problem associated with obesity in horses is laminitis.

Obesity in horses typically becomes established by middle age and is characterized by a cresty neck, excess adipose deposition over the rump and shoulders, and deposition of excess intra-abdominal fat. These horses usually have a history of laminitis, which may be the reason for seeking veterinary care. The onset of laminitis may be insidious in nature, although obese horses also seem particularly prone to acute laminitis precipitated by dietary indiscretion (grain overload, lush green grass). Obese mares often display irregular estrus cycles and infertility. Significantly elevated serum insulin values in such animals is indicative of the insulin refractory state, suggests an increased risk for laminitis, and perhaps a predisposition to PPID.

Hyperlipemia/hyperlipidemia—Hyperlipemia (serum tryglycerides >500mg/dl) is an extreme manifestation of the metabolic consequences of insulin insensitivity. It occurs mostly in ponies, miniature horses, donkeys, mules, and in some horses with PPID, particularly if they are obese. PPID greatly increases the propensity toward hyperlipemia in ponies, miniature horses and obese horses. Ponies and miniature horses are inherently insulin resistant in comparison to other horses. Relative to fit thoroughbreds, for example, fat ponies have elevated resting insulin levels, exhibit an exaggerated insulin response, delayed return to euglycemia in response to glucose loading, and have a tendency toward obesity. Hyperlipidemia (increased serum triglycerides) occurs readily in these animals under conditions resulting in a negative energy balance, such as reduced feed intake due to illness or increased nutritional demand due to pregnancy or lactation. Elevation in plasma triglycerides beyond 500mg/dl is termed hyperlipemia, and is characterized by rapid progression of metabolic acidosis and fatty infiltration of the tissues, most notably the liver. Uncontrolled hyperlipemia leads to a variety of serious physiological derangements. Terminal cases may succumb to a variety of complications including dehydration, shock, severe metabolic derangements, hepatic and/or renal failure, fractured liver, or bleeding diathesis.

Clinical signs of hyperlipemia can include depression, anorexia, ataxia, diarrhea, colic, recumbency, and/or icterus. Signs of hepatic failure, sometimes including encephalopathy, may predominate. The animal is typically obese and has a recent history of illness (such as laminitis), severe stress, or is pregnant or lactating. The plasma is grossly lactescent, which occurs when plasma triglyceride levels exceed 400-500mg/dl. The complete blood count varies depending on the stage of disease and nature of secondary complications. A stress leukogram (neutrophilia, lymphopenia) may give way to leukopenia as gastrointestinal complications (enterocolitis) and generalized debilitation ensue. Metabolic acidosis occurs in association with lipemia and generalized systemic illness, and may be profound resulting in severe depression. Plasma glucose levels may be abnormally depressed or elevated, depending on the degree to which hepatic function is compromised. Elevations in serum bilirubin, hepatic enzyme activities and bile acids occur due to fatty infiltration of the liver; progressive hepatic compromise leads to hepatic failure. Fatty infiltration of the kidneys and concurrent dehydration can cause renal compromise, azotemia and failure. Alterations in serum calcium and electrolyte concentrations occur in association with acidosis, diarrhea and renal compromise. Elevations in plasma triglycerides can be extreme, exceeding 3,000mg/dl in some cases. Extreme elevation in plasma triglycerides is generally a poor prognostic indicator.

A presumptive diagnosis of hyperlipemia can be made based on signalment, history and clinical signs, including lactescent serum. Laboratory evaluation is useful in assessing the degree of hyperlipemia and the metabolic and pathophysiological perturbations associated with the syndrome. Hyperlipemia should be part of the differential diagnosis in any depressed, anorectic, or otherwise sick animal of this class, and serum triglycerides should be included as part of the minimum laboratory database.

Diagnostics for Equine endocrine metabolic disorders—Submit blood and serum for clinical pathology workup (see Fee Schedule in User's Guide or on wet site). For endocrinology submit serum for Total T4 and Cortisol,

Fee=$22 each; Insulin, Fee=$30; submit plasma for Endogenous ACTH, Fee=$55.

ACCREDITATION REQUIREMENT

—Dwayne Hamar

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) has changed the requirements for laboratories to be accredited. The accreditation requirements are very similar to the "OIE Quality Standard and Guidelines for Veterinary Laboratory: Infectious Diseases" from the Office International Des Epizooties.

As of April 1, 2004, all accredited laboratories were required to submit a quality manual and a timeline for implementing the requirements in the quality manual. Full implementation is to be completed by December 31, 2006. Starting in 2007, the accreditation committee will accredit laboratories based on the new requirements. Our next accreditation review is scheduled for the year 2007.

We have submitted our quality manual and a timeline for implementation to the chairperson of AAVLD Accreditation Committee. Within this structure, a quality manager is to have direct communication with the director. Dr. Barb Powers has appointed Dr. Dwayne Hamar as quality manager and Carrie Schmer assistant quality manager. In the near future, quality coordinators for each section and the branch laboratories will be designated.

We have a document in place delineating all of the quality control samples the laboratory routinely uses. Additionally, all sections have good standard operating procedures (SOPs) in their laboratories. The major efforts and challenges for us to comply with the new requirements for accreditation will include creating SOPs for all the activities within the office, training records for all personnel, and communicating the implementation plan with the branch laboratories.

We recognize that implementing these new requirements will take much thought and effort for all employees of the laboratory. We will make every effort to assist and aid employees in this endeavor. We also plan to incorporate as much of the document control and documentation of the quality manual on our employees' website.

An external quality auditor recently informed us that we are doing a quality job but in some areas lacked documentation of this quality. We believe we are well on our way to meeting the new accreditation requirements and continuing to provide you with high quality diagnostic services.

UPDATE ON NATIONAL ANIMAL HEALTH LABORATORY NETWORK

Nearly two years ago, the National Animal Health Laboratory Network (NAHLN) was established. We are one 5-core Animal Diagnostic Laboratories in this system and received funding to upgrade our BSL-3 capabilities and acquire rapid test equipment. The eventual goal is to expand this network to include laboratories in all states. Nearly one year ago, we completed our new BSL-3 modular unit with about 1800 sq ft of laboratory and office space. It is now fully equipped and functional. Gail Chinnook and Dr. Van Campen trained at the National Veterinary Service Laboratory and Plum Island for real-time PCR testing for Exotic Newcastle's Disease, Avian Influenza, Foot and Mouth Disease, Classical Swine Fever, and Vesicular Stomatitis. We are currently conducting proficiency testing for these agents in our new unit. Dr. Mason also recently attended the foreign animal disease course at Plum Island. Jay Kammerzell is leading the Information Technology group of the NAHLN to establish a highly secure but efficient system of laboratory result reporting and data collection. We continue to work with our USDA Area Veterinarian in charge, our state veterinarian, and USDA to help develop a nationwide system designed to protect our agriculture industry.

BSL-3 Modular Unit

HERD TESTING FOR BIOSECURITY

—James Kennedy/Rocky Ford

In this article, we'll review how diagnostic testing applies to a herd biosecurity program. When we look at testing a herd for disease, it is important to understand what sensitivities and specificities, as reported by the Diagnostic Laboratory, mean on a herd basis. The sensitivity of a test is essentially the percent of diseased animals that test positive, while the specificity of a test is the number of negative animals classified correctly as negative. On an individual basis, test sensitivities and specificities are pretty much straight-forward. But when we look at tests on a herd basis, we have additional parameters to consider such as sample size and disease prevalence. In order to evaluate tests on a herd basis, we should use a new set of calculated values. These values include herd sensitivity (HSENS), herd specificity (HSPEC), herd positive predictive value (HPPV), herd negative predictive value (HNPV), herd efficiency (HEFF), and herd apparent prevalence (HAP). Brief definitions of these terms follow:

- HSENS—The probability of correctly classifying an infected herd.
- HSPEC—The probability of correctly classifying an uninfected herd.
- HPPV—The proportion of the test positive herds that have the disease.
- HNPV—The proportion of test negative herds not infected.
- HEFF—The proportion of herds correctly classified.
- HAP—The percent of herds that test positive.

Another point of consideration is how we might sample a herd, with replacement or without replacement of selected animals. As an example, if we place eight white marbles and two black marbles in a hat and draw one out, the probability of drawing a black marble is 20%. If we replace the marble and draw another out, the probability again is 20%. However, if we keep the first marble we drew in our hand and draw another, we arrive at a different set of probabilities. For example, if the first marble was white, then the chance of the second being black would be 2/9. The first sampling scheme with replacement is a binomial probability distribution, while the second without replacement is a hypergeometric probability distribution. More frequently, veterinarians deal with a hypergeometric sampling process. However, if the population of animals you are dealing with is very large, the difference between the two sampling methods becomes quite small and it is easier to approximate the hypergeometric distribution using a binomial probability distribution.

To calculate the probability of correctly classifying an infected herd by hand is quite tedious and it's much easier to use the Excel® Spreadsheet hypergeometric function:

HSENS = 1-hypergeom (0,S,R,H) where we want to find zero reactors from a sample of size 'S,' out of a total possible number of reactors 'R' in a herd of population 'H.' (To accomplish this without the computer requires a series of factorial equations.) As an example, consider a test with 80% sensitivity, 90% specificity, a sample of 15, a prevalence of 10%, and a herd of 100. Solving for R, we take the prevalence multiplied by the herd size multiplied by the test sensitivity (in this case 8), but we also must account for those incorrectly classified as positive, or one minus the specificity multiplied by the quantity of one minus the prevalence multiplied by the herd size (or 9) and a total of 17 reactors. The hypergeometric equation now looks like this, hypergeom (0,15,17,100). The result of this equation is the probability of finding no test-positive animals in the sample. The probability of finding at least one is represented by, 1-hypergeom (0,15,17,100) or 95.2%. By doubling the sample size, we increase the probability of detecting an infected herd to 99.8%. If, on the other hand, we look at a replacement scheme of sampling, we represent the HSENS as, 1-Binomdist (0,15,17,True) and the result is 93.8%. Doubling the sample size again, we arrive at 99.6%. It is interesting to note that a replacement scheme provides a lower estimate of the herd sensitivity. To arrive at the same HSENS as reported using a non-replacement scheme, the sample size needs to increase to 17 head in order to receive the same 95% value as found with the non-replacement scheme and 15 head. The significance of this lies in the cost of testing more samples to achieve the same level of detection.

The next parameter for consideration is the herd specificity of the test (HSPEC). You can calculate this value using the same Excel® Spreadsheet.

In this case, the non-replacement hypergeometric function looks like this – Hypergeom (0,S,F,H), where we want to find 0 false positives, in a sample 'S' size, where we expect 'F' false positives in a herd of 'H' animals. Using the sample above, we can use a specificity of 90%, a sample of 15, and herd size of 100. The values of 'S' and 'H' are self-explanatory. Calculate the value of 'F' by multiplying the herd size by the quantity of one minus the test specificity, or 100 x (1-.9), or 10. This is the number of false positives you might expect in the herd. The formula with all the values in place becomes Hypergeom (0,15,10,100) and the result is 18%. When we look at the replacement scheme, the Bionomist (0,15,10,True) with a result of 20.6% is a less conservative estimate when compared to a non-replacement scheme.

We can use values of HSENS and HSPEC to calculate the herd positive predictive value (HPPV), herd negative predictive value (HNPV), herd efficiency (HEFF), and herd apparent prevalence (HAP), provided we have knowledge of the number of diseased herds (DH). The formulas for these additional measures of herd diagnostic testing are:

$$HPPV = \frac{HSENS \times DH}{[(HSENS \times DH) + (1 - HSPEC) \times (1 - DH)]}$$

$$HNPV = \frac{(1- DH) \times HSPEC}{(1 - DH) \times HSPEC) + (1 - HSENS) \times DH}$$

$$HEFF = HSENS \times DH + (1 - DH) \times HSPEC$$

$$HAP = HSENS \times DH + (1 - DH) \times (1 - HSPEC)$$

If we assign a value of 20% to the number of diseased herds and use the described formulas, we end up with the following results:

HPPV = 22.5%
HNPV = 93.8%
HEFF = 33.5%
HAP = 94.6%

If you ask the laboratory for the sensitivity or specificity of a test, they will give you a value. When that value is applied to a herd basis, the resulting ability of the test to correctly classify the herd is not the same. The greater the HPPV, the

more truly positive herds are correctly identified. The greater the HNPV, the more truly negative herds are correctly identified. The greater the HEFF, the more accurately the test has correctly identified herds tested. The greater the HAP, the higher the percentage of positive herds, although some of those herds are misclassified. Another point to keep in mind is what side of the table you are on. If you are the buyer, you want a test with a high herd sensitivity. If you are the seller, you want a test with a high herd specificity. The example above does an excellent job of identifying positive herds, but not at correctly identifying a negative herd--a buyer's test.

If you wish to discuss these issues more fully, please call Jim Kennedy at Rocky Ford, 719-254-6382.

WHAT'S IN THIS ISSUE OF LABLINES

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