

Colorado State University Veterinary Diagnostic Laboratories



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Letter from the Director


It's been difficult getting this issue of LabLines out the door due to the flurry of activities at the laboratory. In addition to keeping up with growing case submissions and demand for newly available diagnostic tests, we are in the midst of planning for our new building (see inside for details), the Diagnostic Medicine Center. We have new faculty, staff and residents coming on board, and we are conducting searches for two anatomic pathologists. One of these is a brand new position and the other is a vacated position due the impending retirement of long-time diagnostic pathologist Dan Gould.

This past winter in Colorado was a tough one for producers. We tried to assist the cattle industry deal with the consequences of severe winter blizzards by offering discounted abortion and calf diarrhea screening tests. This spring, we were quite active assisting veterinarians and their clients deal with cases of nephrotoxicity in companion animals caused by pet food contaminated with melamine and cyanuric acid. In October, we look forward to hearing the results of the AAVLD pet food toxicity survey at our annual meeting. Thankfully, we are seeing just a few long-term effects of this and hope this remains limited.

The Laboratory now is prepared for a potential outbreak of vesicular stomatitis but we hope this will not occur. This spring, we completed the 2006 Wildbird Avian Influenza Surveillance with USDA Wildlife Services and state wildlife agencies, and are now in the 2007 surveillance season. Chronic wasting disease surveillance in the fall and winter with the Colorado Division of Wildlife has stabilized at about 10,000 tests for hunters in our state.

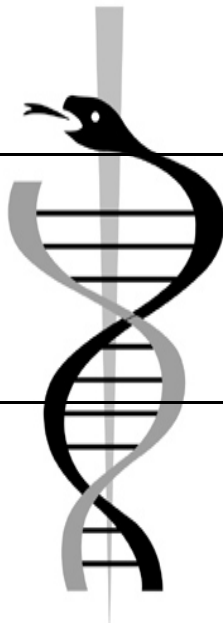
In August, a big event for us is our accreditation site visit by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). This occurs every five years and we are being accredited now to ISO17025 Standards. There is a tremendous amount of "behind the scenes" effort to meet these standards, but the high quality of our diagnostic testing benefits our clients every day.

I hope your summer is going well and look forward to seeing each of you at the Colorado Veterinary Medical Association's annual meeting in September, as well as the AAVLD annual meeting in October.



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Our External Advisory Committee members volunteer their time to meet with us annually and assess our progress, as well as provide input to our future directions. We are grateful for their time and advice, and hope they feel that they are an integral part of the laboratory.

THE NATIONAL ANIMAL HEALTH LABORATORY NETWORK

—Barb Powers

The National Animal Health Laboratory Network (NAHLN) is a federal-state partnership between the United States Department of Agriculture (USDA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The mission of the NAHLN is early detection, response and recovery in case of foreign animal or emerging disease outbreaks.

The NAHLN was created five years ago with five core regional laboratories (of which we were one) and seven satellite laboratories. It now comprises 58 laboratories. However, only the original 12 laboratories receive significant financial support while the other laboratories rely on fee-for-service testing and receive some minimal information technology support.

Since its inception, despite limited funding, the NAHLN has provided surveillance for Bovine Spongiform Encephalopathy (over 800,000 tests), scrapie, Chronic Wasting Disease, Exotic Newcastle Disease, Avian Influenza and Classical Swine Fever. One key to these successful surveillance programs and emergency response readiness is the quality assurance provided by thorough test validation by standardized testing formats, reagents, quality control, and routine proficiency testing.

To further enhance the expertise and proficiency of the NAHLN, a ‘train-the-trainer’ program is in place to expand the ability of trained professionals to perform complex molecular diagnostic tests. High throughput equipment has been installed in many laboratories as well as additional BSL3 space enhanced.

Progress is being made on an information technology (IT) Network to allow rapid and secure data reporting and messaging. We are working on bringing aquaculture into the NAHLN to handle such emergencies as viral hemorrhagic septicemia. We are also working to bring toxicology into the NAHLN, allowing us to better respond to such events as the recent pet food toxicity crisis. Although very successful and effective during the first five years of its existence, the NAHLN remains short of the funding that would allow it to reach its full potential—something we hope will be remedied as the network's value to public health and food safety is fully realized and understood.

VESICULAR STOMATITIS VIRUS COMPLEMENT FIXATION TEST

—Christina Gerhard

Starting 06/15/2007, we will offer a complement fixation test for vesicular stomatitis virus (VSV). The VSV complement fixation test will be available in addition to the serum neutralization test (that we currently offer) as a routine diagnostic test for equine and domestic livestock movement cases.

Additionally, as part of our ongoing involvement in the National Animal Health Laboratory Network, we will be able to use this test in the event of an outbreak of VSV in Colorado and serve as a confirmatory laboratory for diagnosing vesicular stomatitis virus in HORSES ONLY.

Like the serum neutralization (SN) test, the complement fixation (CF) test detects the presence of antibody to VSV in a serum sample. A significant difference between the two tests is that the CF test indicates recent infection. While antibody levels detected by the SN test may remain elevated for months or even years after exposure or infection, antibody levels detected by the CF test begin to decline several weeks after exposure or infection.

Acute and convalescent samples may be submitted if it is thought that the initial specimen was not obtained in the acute stage of the illness.

Please remember that in cases of suspected clinical infection, the state veterinarian or USDA Area Veterinarian-in-Charge must be notified before samples are collected.

All samples will be run at a starting dilution of 1:5 on the CF test unless otherwise specified. For interstate movement, check with the State Veterinarian's Office of the destination state. For international movement, check the test and starting dilution requirements at the National Center for Import/Export's Web site by accessing http://www.aphis.usda.gov/vs/import_export.htm and then clicking on Export Animals Regulations; or contact your AVIC.

The CF test will be run on Mondays, Wednesdays, and Fridays, with results available the same day for samples received by 8AM.

Submit serum (RTT or separator tube). Fee=\$27 (this includes both Indiana and New Jersey serotypes).

REFERENCES:

International and Interstate Testing of Animals for Vesicular Stomatitis Virus Antibodies. Retrieved March 5, 2007 from <http://www.aphis.usda.gov/vs/nvsl/index.html>

Vesicular Stomatitis

What is Vesicular Stomatitis (VS)? Vesicular stomatitis (VS) is a disease that periodically affects horses and cattle in Colorado, and less commonly, sheep and pigs. The disease is characterized by blisters (vesicles) that form on the tongue, in the mouth and lips, teats and the coronary band. The blisters rapidly break open, forming painful ulcers in these locations. As a result, affected animals stop eating, may have a fever, and may be reluctant to move. The disease does not usually cause death in cattle, but can have a negative effect on weight gain and milk production.

What cases VS? The disease is caused by vesicular stomatitis virus (VSV). There are two kinds of VSV in the US – New Jersey (VSV NJ) and Indiana (VSV Ind).

Where does VS come from? The reservoir of these viruses in Colorado remains a mystery. People have speculated that insects, rodents and even plants may be the source. With further study, the source of VS in Colorado will be discovered.

When does VS occur in Colorado? Colorado has had a long history of VS. The first well-documented outbreak and description of the disease occurred in August of 1916 when VS appeared in cattle in the Denver stockyards. Outbreaks of VS have occurred at irregular intervals since 1916. The disease in Colorado is associated with wet springs as occurred in 2004. The first VS cases are usually observed in late June and continue through the summer.

Why is VS important? Vesicular stomatitis is important because cases in cattle and pigs can have some resemblance to important foreign animal diseases like foot-and-mouth disease (FMD). Since FMD has more severe implications for US animal agriculture and trade, it is important to investigate similar diseases to determine the exact cause.

The presence of VS cases in Colorado also affects interstate and international animal movement and trace. For example, horses are not infected by FMD viruses. However, when horses develop VS, quarantine and interstate transport restrictions are put into action which restricts sporting events such as horse shows and rodeos.

What do I do if I observe sores or blisters in the mouths of my livestock? Call your veterinarian. With assistance from the Colorado state and federal veterinarians, your veterinarian will obtain samples for diagnostic testing to determine whether animals are infected with VS. In doing so, you will help keep US animal trade on course.

By performing complement fixation (CF) and serum neutralization (SN) tests for VSV antibodies, Colorado State University's Veterinary Diagnostic Laboratory helps support VS control measures in Colorado.

PHENOBARBITAL-ASSOCIATED CYTOPENIAS--CLINICAL AND CLINICOPATHOLOGIC FINDINGS IN 3 DOGS

--Davis Seelig and Christine Olver

Phenobarbital (PB), a barbiturate used primarily as an anti-seizure medication and sedative, is generally considered a fairly safe and effective drug. Reported adverse effects in the dog vary widely in significance and range from the relatively benign (increased serum liver-associated enzymes, alteration in thyroid hormone profile, polydipsia, polyphagia, and polyuria) to the more severe (hepatotoxicity, superficial necrolytic dermatitis, bone marrow necrosis, neutropenia, and thrombocytopenia). This report details the clinical, hematologic, and bone marrow cytologic findings in three dogs with phenobarbital-associated cytopenias and bone marrow dysplasia (dysmyelopoiesis).

Samples submitted to the Clinical Pathology laboratory at Colorado State University (CSU) for complete blood count (CBC) and bone marrow aspiration cytology were from three dogs. Two of the dogs were presented directly to the Veterinary Teaching Hospital and one was presented to a referral veterinarian. In the latter dog, the samples were submitted to the Clinical Pathology laboratory through the Diagnostic Laboratory.

All three dogs were receiving appropriate dosages of PB for presumed idiopathic epilepsy (prescribed dose: 1.7–4.0mg/kg BID, recommended dose: 1–4mg/kg BID–TID). The dogs had been receiving PB therapy for an intermediate period of time (2.5 to 6 months) and in all dogs, seizure activity appeared to be under

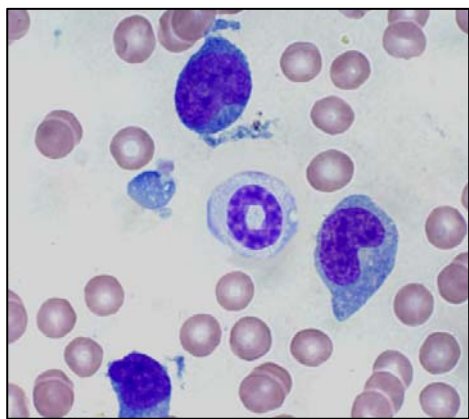
control (Table 1). All three dogs presented to their respective veterinarians with a history of either lethargy (2/3 dogs) or weakness (1/3). Initial workup of each of the three dogs included, among other diagnostics, a CBC.

Case	Signalment	PB Dose	Duration of PB Administration	CBC Findings ($\times 10^3/\mu\text{L}$) ^a
Dog 1	7 y/o ♂(n) Dachshund	2.0 - 4.0 mg/kg BID	6 months	Leukopenia: 5.3 Neutropenia: 2.0
Dog 2	6 y/o ♀(s) Mix	1.7 mg/kg TID	3 months	Leukopenia: 1.8 Neutropenia: 0.8 Thrombocytopenia: 59
Dog 3	3 y/o ♂(n) Vizsla	2.9 mg/kg BID	2.5 months	Leukopenia: 1.6 Neutropenia: 0.0 Thrombocytopenia 38

^a CSU reference ranges: Nucleated cells: $6-17 \times 10^3/\mu\text{L}$, neutrophils: $3-11.5 \times 10^3/\mu\text{L}$, and platelets: $200-500 \times 10^3/\mu\text{L}$

In all of the three dogs, evaluation of the CBC revealed a decrease in the concentration of one or more of the marrow-derived peripheral blood populations. Moderate to severe neutropenia (3/3) and thrombocytopenia (2/3) were the most common hematologic abnormalities (Table 1). One of the three dogs had a mild, normochromic, normocytic, non-regenerative anemia (interpreted as anemia of chronic inflammatory disease). In the face of a persistent (repeatable more than once over a 48-hour period) neutropenia with no obvious source of peripheral consumption, all three dogs underwent bone marrow aspiration. The cytology samples subsequently were submitted to the Clinical Pathology laboratory at CSU for evaluation.

Cytologic evaluation of bone marrow aspirates revealed samples which were of normal (1/3) to increased (2/3) cellularity with adequate (2/3) to increased (1/3) numbers of megakaryocytes, and a normal erythroid lineage. In all three dogs, the myeloid lineage was extremely left shifted, characterized by an abundance of myeloblasts, promyelocytes, and metamyelocytes. In two of the three dogs, there was morphologic evidence of granulocytic dysplasia which was characterized by a combination of asynchronous maturation and a variable number of ring granulocytes (Figure 1).



The microscopic features of the bone marrow were indicative of a condition known as dysmyelopoiesis (DMS), which is defined as a disorder characterized by the presence of dysplastic cells in the blood or bone marrow. DMS is further classified into clonal (myelodysplasia) and non-clonal (secondary and congenital) etiologies. In veterinary medicine, conditions associated with secondary DMS, considered the most commonly identified form, include immune-mediated hematologic disease, lymphoma, myelofibrosis, and drug toxicity. This report is the first to detail the cytologic findings and myelodysplastic features of phenobarbital-associated myelotoxicity and strengthens previous reports of PB-associated neutropenia and thrombocytopenia. Finally, based upon these findings, we propose an immune-mediated mechanism to these cytopenias. In such a

scenario, the resultant dysplastic changes in the granulocytic lineage are the result of intense, compensatory hyperplasia.

Following discontinuation of phenobarbital treatment, neutrophil and platelet numbers normalized in two of the dogs (one dog lost to follow-up). This normalization of hematologic parameters following cessation of

PB administration, in conjunction with attempts to rule out other causes of neutropenia (3/3 dogs lacked titers to *Ehrlichia canis*) and a previous published report of PB-associated cytopenias, supports PB toxicity as the underlying cause. Although serum concentrations of PB were not determined, the prescribed dosages were considered appropriate according to the current literature.

On the basis of these cases, the following conclusions are presented:

- Early hematologic evaluation (within six months) of dogs started on PB is recommended for the detection of possible myelotoxicity.
- The toxic effects of PB are reversible and resolve rapidly (two-eight weeks) following drug cessation.
- In cases of unexplained cytopenia(s), bone marrow aspiration and cytology are useful diagnostic tools.
- PB toxicity should be considered in dogs with unexplained cytopenias or morphologic evidence of dysmyelopoiesis.

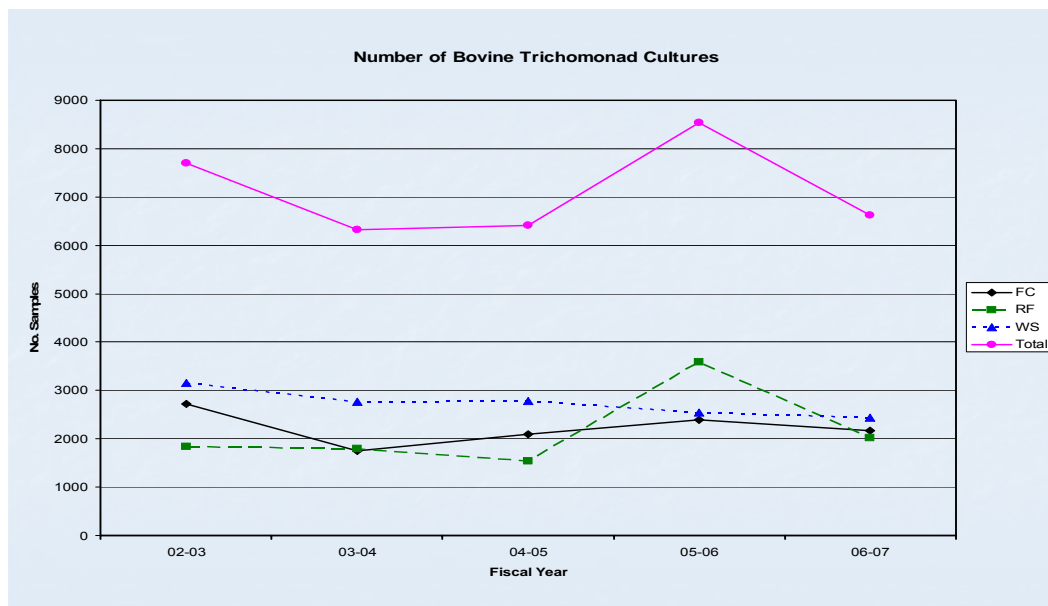
CBC and bone marrow cytology--Submit bone marrow aspirate and 1cc blood. Fee=\$53.50.

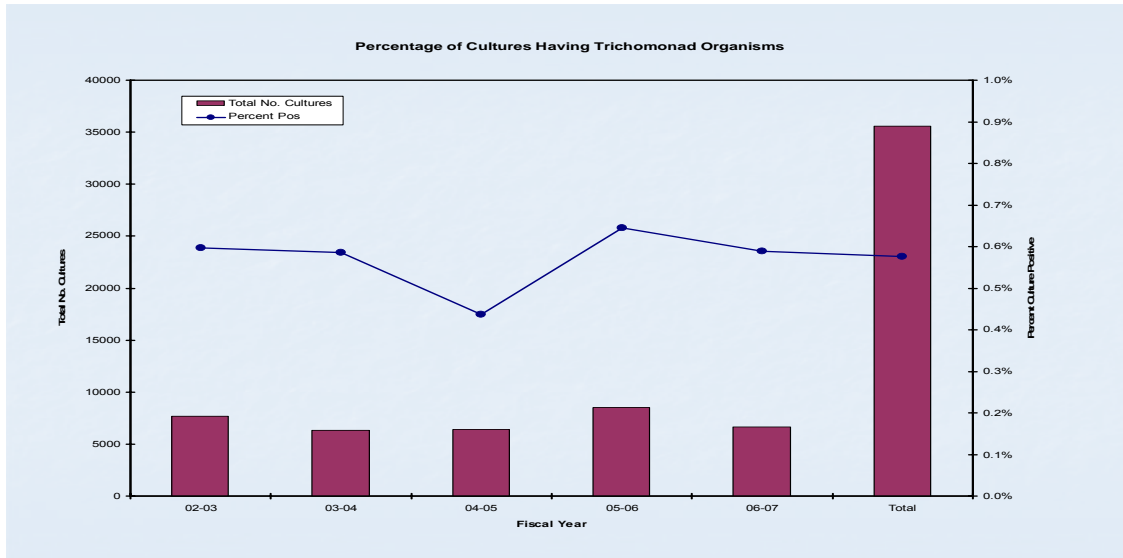
ON-LINE ACCESS TO RESULTS--Tired of waiting for that fax? Would on-line access to your results be useful? Email Carrie at cschmer@colostate.edu. She will create your account so you can see your results as soon as they are complete and at your convenience, day or night.

5-YEAR SUMMARY OF BOVINE *T. FOETUS* CULTURES

—Lora Ballweber

Over the last five years, we tested between 6,000 and 9,000 cattle samples annually for *T. foetus*. Our Western Slope Laboratory tends to have the highest number of samples, however, in FY05/06, a spike of increased testing occurred as a result of increased samples at our Rocky Ford Laboratory. The percentage of culture positive samples remains less than 1% over the last five years. The culture does not distinguish between *T. foetus* from non-pathogenic trichomonads, so PCR is used to confirm the culture results and identify *T. foetus*.





T. foetus testing—Submit preputial wash in lactated ringer's or In-Pouch TF (Biomed Diagnostics). If samples are collected in lactated ringer solution, samples must reach us within 24 hours after collection. Samples taken in In-Pouch TF pouches should reach us within 48 hours after collection. Fee=\$6 (1-50); \$5 (51+).

BLUETONGUE CASES IN SHEEP FLOCKS OF NORTHEASTERN COLORADO

--Christie Mayo and Richanne Lomkin

Field investigations performed during the fall of 2006 revealed several positive bluetongue sheep flocks along the South Platte River. Clinical signs included lameness, wool loss, weight loss, oral ulcerations, and edematous muzzles and ears. Morbidity was observed predominantly in lambs and they were treated with supportive care and Banamine® to reduce the pain secondary to coronitis. The virus prevalence of the herds sampled was 40 percent supporting bluetongue as the probable cause of clinical signs observed.

Bluetongue (BT) is caused by bluetongue viruses (BTV) transmitted by *Culicoides* spp. gnats. Bluetongue viruses are arthropod-borne and belong to the family Reoviridae, genus *Orbivirus*. Since the first isolation of BTV in the United States in California sheep in 1953, 24 serotypes have been identified worldwide of which four (10, 13, 11, and 17) are commonly isolated in the United States. It is a reportable disease and, while infected cattle do not typically develop clinical signs of disease, sheep can develop significant morbidity. Clinical signs typically include weight loss during acute infection, decreased wool production due to "wool break," lost income from ram sales due to interstate trade restrictions on BTV-test positive animals, and decreased reproductive viability due to consequences of *in utero* infections and transient ram infertility.

The OIE's (Office of International Epizootics) Terrestrial Manual designates three classifications of BTV status based on animals within latitude boundaries of 53°N and 34°S. This manual regulates transportation and free trade of animals based on BTV-free zones, BTV-seasonally free zones, and BTV-infected zones. Colorado is currently classified as seasonally free of BTV; however the epidemiology of BTV appears to be changing. Recent BTV outbreaks in northern Europe have caused significant morbidity in cattle as well as sheep, and serotypes previously found primarily south of latitude boundary 53°N have recently migrated northward possibly due to global climate changes and increased vector range.

Although ruminants are capable of transmitting BTV for no more than 60 days post-infection, a multitude of states with BTV-free status deny transport of animals based on antibody detection methods. Antibodies can

be detected as early as 14 days post-infection, but can persist for long durations in the field, thus creating an inadequate measurement of active infection. These factors support a need to understand the epidemiology and evolution of the virus because interstate and international trade restrictions continue to be one of many economic concerns impacting Colorado's sheep industry during seasonal ram sales and shows.

Colorado's sheep industry contributes \$96 million annually to Colorado's cash farm receipts and is ranked fourth in the nation for total sheep and lambs produced annually, but little is known about seroprevalence within Colorado. Therefore, rational control measures for the disease are difficult to develop or recommend. Currently, there is an initiative to understand these factors so that producers, veterinarians, consumers, and the general public can work together in order to further understand the epidemiology of this disease and its economic importance. This effort will be headed by Drs. Ashley Hill and Christie Mayo at Colorado State University. Flocks that test positive by PCR and have concurrent clinical signs should be reported to the USDA veterinary service area office. Further information and inquiries concerning current projects should be directed to Dr. Christie Mayo (contact information below).

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Fee: BTV AVID Serology—Submit 1ml of serum. Fee=\$5. BTV PCR—Submit 4ml unclotted blood or spleen. Fee=\$30. BTV Virus isolation—Submit 10ml of whole blood. Fee=\$40.

UPDATE ON EQUINE VIRAL ARTERITIS SEROLOGY

—Hana Van Campen

Colorado's equine viral arteritis (EVA) testing program is in full swing. To accommodate the interest in this program, we offer two options for EVA serology.

- EVA screen (Test #832)—This test determines whether there is EVA-neutralizing antibodies present in a single serum sample. The results are reported as “positive” or “negative.” To-date, most Colorado horses have not been previously infected or vaccinated, and do not have EVA antibodies. This test is useful to screen stallions before they are vaccinated or mares prior to shipment to breeding farms.
- EVA SN serology (Test #822)—This test estimates the quantity of EVA antibodies in serum and a titer of <1:4 to 1:512 will be reported. This test is best used:
 - a. To demonstrate an increase in EVA SN antibodies between acute and convalescent serum samples in suspected cases of EVA abortion or other EVA-associated disease.
 - b. To confirm an EVA specific immune response in stallions that have previously tested positive for EVA antibodies with the screening test.

Both EVA serology tests are set up twice a week – Tuesday with the results reported the following Friday evening, and Friday with results reported the following Monday evening. Serum samples should be shipped so that they are received the day before tests are set up.

EQUINE VIRAL ARTERITIS (EVA)

Equine viral arteritis virus belongs to a unique virus family, the Arteriviridae, whose only other virus of veterinary importance is porcine reproductive and respiratory syndrome (PRRS) virus.

In horses, the diseases caused by EVA virus (EAV) have been recognized for over one hundred years. EAV infections result in respiratory disease including pneumonia in foals, damage to blood vessels which results in fluid in tissues (edema) and abortions.

The initial infection is through the nose and upper airways where the virus enters the blood and spreads to all tissues. All of the members of this family have an affinity for macrophages, a central cell of the immune system, and endothelial cells, the cells that line blood vessels

Infection of the blood vessels damages them leading to edema and bleeding. In severe cases, the damaged blood vessels causes blood clot formation and loss of blood flow to internal organs. The virus readily crosses the placenta and infects the fetuses of pregnant mares causing tissue damage. The death of the fetus ends in abortion.

In the intact colt or stallion, EAV infects the testicles and accessory glands. More importantly, the stallion may become a carrier of EAV and shed infectious virus in semen. Breeding or artificial insemination of mares with the semen from carrier stallions leads to infection in the mare. While the mare may not show any signs of disease, she will shed infectious EAV from her respiratory tract and infect other mares. This scenario has led to multiple EVA abortions on individual farms.

To determine whether horses have been infected with EAV, serum is tested for antibodies (SN) specific for EVA virus. Horses carry EVA SN titers for life. EVA antibodies in mares, fillies and geldings simply indicate past infection with the virus. However, stallions with EVA antibodies require PCR testing or virus isolation of their semen to determine whether they are carriers.

In 2005, semen from two EVA-infected quarter horse stallions was shipped to a number of veterinary clinics and reproductive centers in the US. In addition, mares bred to these stallions were shipped to premises in many states. Several farms experienced clinical disease including respiratory infections and abortions.

Investigations or "trace backs" of bred mares were performed by Colorado's state veterinarians and showed that luckily, EVA-infected horses did not reach Colorado.

In Colorado's EVA prevention program, stallions are tested for EVA antibodies 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.

Since July 1, 2006, Colorado State University's Diagnostic Laboratory has performed 5,479 EVA SN tests.

Colorado's EVA control program has been enthusiastically embraced by horse owners in Colorado and surrounding states. Universal participation in such voluntary programs will help keep Colorado's horse population healthy.

EVA Screen—Submit 1ml serum. Fee=\$7. EVA SN—Submit acute and convalescent serum. Fee=\$11.

HAVE YOU EVER HEARD OF . . . METATARSAL FISTULATION OF THE GERMAN SHEPHERD DOG —Debra Kamstock

A surgical biopsy specimen, obtained from a draining tract near the footpad of a 4-year-old male, intact, German Shepherd dog, was submitted to the laboratory for evaluation. The submitting clinician provided a thorough clinical history which indicated a two-year duration of a chronic draining wound from the **left** metatarsal region just proximal to the metatarsal pad. This lesion had been previously biopsied and diagnosed as chronic, hyperplastic, ulcerative, pyogranulomatous dermatitis. Special stains for fungi and

acid-fast bacteria were performed with negative results. The dog was treated with various courses of antibiotics at which time the lesion seemed to improve yet never completely resolved.

The current biopsy specimen submitted to the laboratory was obtained from a similar lesion now developing in the **right** metatarsal region, again just proximal to the metatarsal pad. Upon microscopic examination, there was similar, extensive and severe, pyogranulomatous inflammation of the dermis and subjacent panniculus with necrosis, clefting, and tract-like formation. The overlying epidermis was hyperplastic and ulcerative, again similar to the microscopic findings two years prior from the lesion of the opposing limb. Special stains for fungal agents and acid-fast bacteria were pursued again with negative results and no foreign body material was identified. Culture did reveal a heavy growth of both hemolytic and non-hemolytic *Escherichia coli*, as well as *Staphylococcus aureus*. However, while these agents were likely contributing to the observed inflammation, they were interpreted as secondary rather than primary pathogens.

Considering the signalment, clinical history, bilateral involvement, and lack of any identifiable primary inciting cause for the lesions, there was a high suspicion for a possible breed-specific idiopathic condition. *Skin Diseases of the Dog and Cat*, 2nd ed. was referenced and the entity *Metatarsal fistulation of the German Shepherd dog* also known as *sterile pedal panniculitis* and *deep metatarsal/metacarpal toritis* (inflammation of the footpad) was identified. It was a perfect match.

Metatarsal fistulation of the German Shepherd dog is considered an uncommon dermatopathy which is characterized clinically by well demarcated, often symmetric, deep fistulous tracts of the proximal aspect of single or multiple pawpads (Fig. 1 - Olivera AM, et. al 2006). The metatarsal pads are affected most commonly followed by the metacarpal pads and, very rarely, other paw pads. The etiology is, as of yet, undetermined however the breed predilection for the German Shepherd suggests heritability. Dogs also may present with concomitant perianal fistulas and / or interdigital bacterial furunculosis, conditions to which German Shepherds also are predisposed. An increased frequency has been noted in males as compared to females and adult dogs from 2-to-8-years old predominate. While the syndrome is primarily seen in German Shepherds, cases in German Shepherd cross breeds (Kunkle 1993) as well as a single case in a Weimaraner (Oliveira 2006) also have been reported.

Differentials for this condition are few due to the unique clinical presentation. Fistulous tracts subsequent to foreign body material should be considered although the anatomical location of the lesion at the proximal portion of the footpad is a bit atypical for a penetrating foreign body. While clinical differentials are few, the microscopic features of hyperkeratosis, acanthosis, ulceration, marked pyogranulomatous dermatitis, and panniculitis, with or without the presence of tract formation depending on the location of biopsy acquisition, are fairly non-specific. These changes could be associated with deep bacterial or fungal infections, a foreign body reaction, or a traumatic penetrating wound. Thus, in the absence of salient clinical information including, at the forefront, signalment and anatomical location, the appropriate diagnosis of metatarsal fistulation of the German Shepherd dog could go undetermined.

In summary:

1. If you are a clinician presented with draining fistulous tracts of single or multiple foot pads in a German shepherd dog think *metatarsal fistulation of the German Shepherd dog*
2. If you are a pathologist presented with tissue from a draining fistulous tract of single or multiple foot pads in a German Shepherd dog think *metatarsal fistulation of the German Shepherd dog*
3. As microscopic lesions often may be non-specific, the provision of thorough clinical information may be *critical* in deriving the correct diagnosis such as with *metatarsal fistulation of the German Shepherd dog*. And. . . last but not yeast,
4. Now you *have* heard of **metatarsal fistulation of the German shepherd dog**

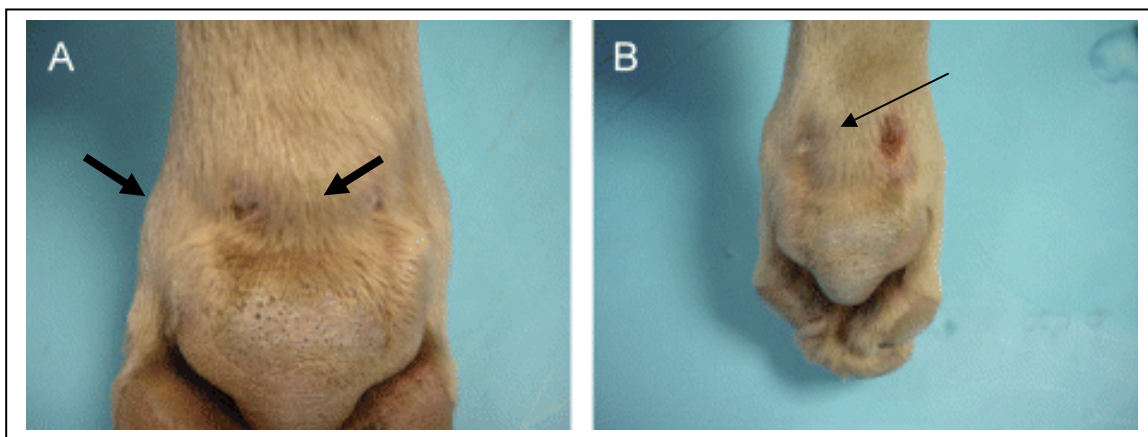
Thank you to Dr. J. Flick at Flatirons Veterinary Specialists for the biopsy submission in this interesting and uncommon case, and for her thorough clinical history which assisted us in determining the diagnosis of metatarsal fistulation of the German Shepherd dog.

References:

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A. Two symmetrical fistulae on the plantar aspects of the left metatarsus with proximal swelling. **B.** Similar lesions on the plantar aspect of the right metatarsus with serosanguinous material discharging from the lateral fistula. (Oliveira AM, et al 2006)

UPDATE ON SCRAPIE DISEASE IN SHEEP AND GOATS IN THE UNITED STATES

—Roger Ellis/Extension Vet

Since 1952, the USDA/Veterinary Services/Animal and Plant Health Inspection Service has been active in the surveillance and control of Scrapie in sheep and goats in the United States. An accelerated scrapie eradication program was initiated in 2000. Primary aspects of this program are animal identification, surveillance, tracing positive and exposed animals, testing of sheep and goats in exposed flocks, clean up of infected flocks, and certification of flocks. In 2005, a key action item of the National Animal Health Surveillance System focused primarily on the effectiveness of the Regulatory Scrapie Surveillance Program testing component and other non-slaughter surveillance testings since 2001. The Animal Health Report of 2005, issued by the USDA-APHIS-VS-CEAH in August 2006 contained some interesting points concerning the US sheep and goat industries and scrapie control statistics.

The estimated sheep and lamb inventory within the US for 2005 was 6.2 million upon 68,280 operations. Colorado has an estimated 400,000 sheep and ranks 4th in the nation for total sheep and lambs produced

annually. The estimated goat inventory was 2.8 million. More than 90 percent of the identified operations had less than 100 total animals and accounted for nearly 29 percent of the inventories. Sheep and/or goat premises recorded in the national scrapie program database totaled 103,580, and 73,807 of these premises have implemented animal ID practices of official tagging standards.

The surveillance and testing of adult sheep and goats at slaughter (Regulatory Scrapie Surveillance System (RSSS)) identified 106 positive animals from 30,247 sample submissions. These samples were collected from 78 sites in 24 states. Of the positive samples, 93 were black-faced sheep, 11 were mottled-faced sheep, one was a white-faced sheep and one was of unknown type. Follow up of these identified positive cases and other regulatory field testing included an additional 5,200 tests for scrapie. From the regulatory field testing, 165 newly identified infected flocks were found and 598 positive animal cases, including two goats. A summary of scrapie cases from 2003 to 2005 is included in the following table.

Scrapie Cases, FY2003 to FY2005

Tests or examinations	Number of Positive Cases		
	2003	2004	2005
Necropsies	315	374	461
Regulatory third-eyelid	32	20	31
RSSS	23	86	106
Total	370	480	598

From: US Animal Health Report 2005

As part of the National Animal Health Laboratory Network, we perform the immunohistochemistry test for scrapie through the RSSS. We perform approximately 8,000 IHC per year for this program, though USDA funding.

GET TO KNOW THE LABORATORY--WELCOME to Dr. Tawtik Aboeilail, New Anatomic Pathologist Faculty Member



Dr. Aboellail earned his bachelor and master degrees in veterinary medical sciences from Cairo University, Egypt, and finished his PhD at South Dakota State University in a joint program between the two universities. Following a three-year residency at Kansas State University, in 2004 he passed his board certification examination in anatomic pathology. Dr. Aboellail worked as a clinical instructor at Washington State University and as head of diagnostic pathology at the Texas Veterinary Medical Laboratory before joining CSU. His research has focused on the pathology of food animals, with special emphasis on bovine and camelid perinatal mortalities. Dr. Aboellail was a member of a team of Egyptian and US researchers who isolated, typed, and described the pathologic picture of BVDV infection in dromedary camels. He also brings with him 15 years of experience teaching veterinary pathology to both undergraduate and graduate veterinary students.

ROCKY FORD EXPANSION COMPLETED

Last October, the Rocky Ford Branch Laboratory began construction on an 1,100 sq. ft. addition to the 1960s vintage existing facility. After a winter unfavorable to construction, the addition was completed in March, just in time for the spring run. The addition includes three new lab rooms plus a small conference room. Along with the additional space, a new peaked roof replacing the old flat roof was added along with a new heating system including central air conditioning. The new roof has dramatically improved the curbside appearance of the facility, and has been greatly appreciated by the staff and community. With the new

facilities, the laboratory plans on adding some additional tests and expanding the current volume of tests performed. The laboratory is currently providing testing service, predominantly in the beef cattle area, for a diverse set of clients across the United States and Canada.

Addition completed in March, just in time for the Spring rush



Rocky Ford's New Personnel



Dayla Pearl is our new technician, Dayla's main area of concentration is PCR testing.



Laura Mendenhall is our newest hire. Laura will be receiving cases, she is where it all starts. Laura was hired after Peggy retired in January of this year.

On a health note, the winter storms took their toll on livestock and people in the area. Although there were a number of livestock deaths directly related to weather, the long-term effect continues. Weak calves and abortions have been a common sequel to the stress from the storms. The inability to provide adequate quality nutrition for near-term cows resulted in poor colostrum production and retained placentas. Feed became scarce and much of what was available was of only marginal value. It would be reasonable to expect the winter problems to continue into the fall with increased health problems in calves due to inadequate passive transfer from poor colostrum intake and production. We also expect an increased number of open cows due to the lower nutritional plane as we go into the breeding season. Despite all the gloom and doom, the sun is shining, the pastures are looking lush and we are looking forward to a bumper year.

We would like to invite any and all to come by, see the new laboratory, and meet the staff.

CHANGES AT THE WESTERN SLOPE VETERINARY DIAGNOSTIC LABORATORY

—John Andrews

During the past year there have been several major changes at the Western Slope Veterinary Diagnostic Laboratory (WS VDL) aimed at improving services. The Western Slope VDL is a branch laboratory of the Colorado State University Veterinary Diagnostic Laboratory System and is located in Grand Junction, Colorado. The facility was constructed in the mid-1970s and was operated by Dr. Darrell Schweitzer until his retirement in July 2005. In May 2006, Dr. John Andrews became the Director of the WS VDL.

Since all the services of the main laboratory in Fort Collins are available through this branch laboratory in Grand Junction, we often operate as a triage laboratory, where, on a daily basis, we process specimens for shipment to the main laboratory as well as examine them locally. Since the full power of the main laboratory at Colorado State University is available and there is excellent overnight delivery service to Fort Collins, it is not necessary or economically viable for all services to be provided locally. Diagnostic services provided locally must, therefore, reflect a combination of immediate local needs, available expertise, and economic feasibility. The services and expertise provided at the WS VDL include pathology and histopathology, general microbiology, and selected serology and agent detection methodologies.

One of our major goals of the past year has been to update the facility and upgrade equipment at the laboratory. Both have been improved to reflect modernization of the services provided. The space available for necropsy examinations has been expanded and the necropsy room redesigned to improve functionality, with walls and floors resurfaced, and overhead lighting brightened. In the histopathology laboratory, we replaced nearly all its processing equipment. Major new pieces of equipment include a closed-system vacuum tissue processor and an improved embedding center which has enabled us to markedly improve the quality of histologic sections. New freezers and refrigerators have expanded specimen storage capacity.

Digital camera equipment for use in the post-mortem room, as well as a digital camera attached to a new modern pathology microscope, has enabled us to consult on cases with other pathologists using the Internet. Other new modern tools such as computer-based transcription software for report preparation has assisted in reducing turnaround time on pathology cases.

Pathology services are provided by Dr. John Andrews, who also serves as the laboratory director. Dr. Andrews is certified in veterinary pathology by the American College of Veterinary Pathology, holds a DVM degree from Iowa State University, and a PhD degree in veterinary pathology from Michigan State University. He has more than 35 years of experience in teaching pathology and providing veterinary diagnostic services. He has previously served at the Iowa State University Veterinary Diagnostic Laboratory, University of Missouri Veterinary Medical Diagnostic Laboratory, and the University of Illinois Veterinary Diagnostic Laboratory. Current pathology services include full and complete necropsies, and histopathology examinations on everything from the necropsy cases to surgical biopsies. Reflecting the animal population of western Colorado, the majority of the necropsy cases are cattle, sheep, other small ruminants, horses, backyard poultry and a variety of wildlife with lesser numbers of dogs, cats and camelids. The majority of the biopsy specimens are from dogs, cats and horses. Histologic examinations of food animal cases originating from the CSU Rocky Ford branch laboratory are often performed at the WS VDL. Dr. Andrews is available for telephone consultation with veterinarians and brings with him considerable experience and expertise in swine diseases, in addition to his experience with the above mentioned species.

With a large equine population on the Western Slope of Colorado, we perform equine infectious anemia (Coggins) serology nearly every business day. Monday through Thursday, samples received by 4PM are set up for Coggins testing and reported by late afternoon (24 hours) the next day. Friday samples are reported on Monday. During the breeding season, we support the area practitioners by processing uterine cultures.

In support of the numerous cattle ranches on the Western Slope, we continue to offer a local service for *Tritrichomonas fetus* detection in breeding bulls (InPouch TF®). Suspect *Tritrichomonas fetus* cultures are sent to the Fort Collins VDL for confirmation by molecular methods (PCR).

The WS VDL has been actively involved in performing and improving the *Brucella ovis* ELISA serology method for the detection of this agent in breeding rams. Because of our national reputation for performing this procedure, we receive sera from nearly all states. We have been involved in recent improvements in this procedure in collaboration with the National Veterinary Services Laboratory and other laboratories across the United States. We reported on the improvements in this procedure at the annual meeting of the US Animal Health Association in Minneapolis, MN in October 2006. Since that time, the test has been further refined and is performed at the WS VDL every Wednesday and more often as needed. The newest version of the ELISA serology for the detection of *Brucella ovis* infection in sheep is more sensitive and specific than the methodologies used in the past. With the latest version of this procedure, there has been a marked reduction in the troublesome non-specific or background reactors previously seen in young ram lambs.

During the fall and winter months, we assist the Colorado Division of Wildlife by providing ELISA testing for the detection of chronic wasting disease (CWD) in hunter-harvested cervids for the western one-third of Colorado.

With the expanded activity, the laboratory also has expanded its staffing. While Dr. Andrews provides the pathology services, Kim Hannafious, the lead technician, provides and coordinates the microbiology services, *B. ovis* serology, Coggins testing and *Tritrichomonas fetus* culturing. Technician Cheryl Thomas is primarily responsible for histopathology processing, assisting the pathologist with necropsy examinations, performing parasitology examinations and working with Kim Hannafious to perform *B. ovis* serology, Coggins testing and *Tritrichomonas fetus* culturing. During the fall and winter months, both Kim and Cheryl, along with seasonal part-time employees, perform the CWD ELISA testing. Paula Hammons serves as the administrative assistant and is complemented in that role by Toonie Histia.

Several major changes in the city of Grand Junction have made access to the laboratory much easier. With the completion of the 29 Road bridge over the Colorado River in July 2006, and the partial completion of the Riverside Parkway (D Road), access to the WS VDL from the south east or west has markedly improved. The laboratory is located at 425 29 Road, just one quarter mile north of the intersection of 29 Road and D Road (Riverside Parkway). Within the next few years the city of Grand Junction plans to extend 29 Road north to Interstate 70. This should also improve access to the laboratory from the north and Interstate 70.

Improved access to the laboratory and the surrounding area also may have an impact on the future location of the WS VDL. Currently, the section of land on which the laboratory is located is leased from Mesa State College. Mesa State College is seeking to re-zone the entire section to allow both commercial and residential development.

We look forward to continuing to serve the CDOW, the veterinarians and animal owners of western Colorado with locally performed laboratory examinations and as a means to tap into the vast expertise of the main VDL in Fort Collins. Please stop by and visit us whenever you are in the area.

The Western Slope Veterinary Diagnostic Laboratory can be reached by phone at 970-243-0673 or by fax at 970-242-0003.

PREVIEW OF THE NEW DIAGNOSTIC MEDICINE CENTER

Planning for the new Diagnostic Medicine Center (DMC) is underway! This new, 90,000 sq ft, three-floor building will be located immediately north of the current Veterinary Teaching Hospital in Fort Collins. There will be an enclosed connection to the hospital as well. Construction is to begin in October 2007 with an anticipated completion date of December 2009. We are very excited about this project as we have long ago outgrown our current space. We thank all of you at the Colorado Cattlemen's Association, Colorado Veterinary Medical Association, Colorado Horse Authority and our External Advisory committee for support of our needs and we thank the state legislature for funding the project.



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