Colorado State University Veterinary Diagnostic Laboratories

LabLines

Volume 5, Number 1 **Fall 2000**

Letter from the Director

We have already had one of those tree-breaking snowstorms in Fort Collins and it's not even winter yet! Fall brings another issue of LabLines that we hope you enjoy. We successfully ended the 99/00 fiscal year on June 30, 2000 with further increases in accessions over the year before, and a balanced budget. We are starting this fiscal year with many changes. We have a new modular unit on the north side of the Veterinary Teaching Hospital and moved our office and sample entry area into it. The office staff are enjoying windows! The space they vacated has been remodeled into expanded laboratory space for the microbiology sections, especially for molecular diagnostics. This modular unit is a temporary measure and we hope to have a new facility in the not too-distant future.

We have successfully completed our search for a new virologist and are pleased to welcome Hana Van Campen, DVM/PhD, to head our Virology Section. Dr. Van Campen, who will join us in January, is a nationally-recognized expert on BVD in cattle.

We were happy to see many of you in Snowmass at the annual Colorado Veterinary Medicine Association meeting. I personally want to thank you for your support in choosing me as Secretary/Treasurer Elect for the CVMA. I am honored to serve the CVMA in this capacity and look forward to seeing you in January at the annual conference.

As always, we strive to provide you with quality and timely service and are continually adding new tests or new services to meet your needs. Please contact us any time if you have suggestions or ideas to improve our services to you.

Barbara E. Powers

Barb Powers, DVM/PhD

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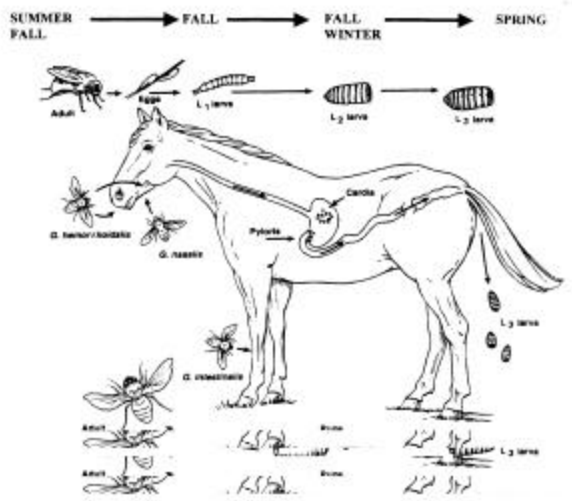
FALL AND WINTER ARE TIMES TO TREAT EQUIDS FOR BOTS

John C. Cheney

Bots are the larval stages of botflies (*Gasterophilus*). Two species, *Gasterophilus intestinalis* (the common bot) and *Gasterophilus nasalis* (the throat bot) are very common in equids in the United States. An additional species, *Gasterophilus haemorrhoidalis* occurs rarely in this country. All ages of equids can be infected with horse bots.

To treat and control bots in equids, it's necessary to understand the developmental cycle of this parasite. Adult flies are large, robust, and bee-like in appearance. The adult fly does not feed and only lives long enough to mate and deposit eggs on the hairs of the equid, after which the adult fly dies. Flies actively deposit eggs during the warmer months of the year. In the Rocky Mountain states, this is from mid-summer to first frost in the fall. The common bot lays eggs on the hairs of the forelegs and shoulders, whereas the throat bot lays its eggs on the intermandibular hairs. The eggs are seen easily being 1.0-2.0mm long and usually creamy white. Larval stages of the common bot may remain viable in the eggs for a prolonged period of time, up to 100 days. Eggs of the common bot require moisture and a stimulus to hatch, which occurs when the horse licks at the area where the eggs are attached. Newly hatched larvae are carried to the mouth by the tongue. Eggs of the throat bot hatch spontaneously and crawl into the mouth. Larvae then penetrate the tongue or bucal mucosa and wander in these tissues for about a month. Larvae then pass via the pharynx and esophagus to the stomach where they attach to the gastric epithelium for the winter. Larvae remain and develop in this site for periods of eight-10 months and then mature in the following spring or early summer. They then detach and are passed in the feces. Pupation of the larvae takes place on the ground and, after one to two months, the adult flies emerge.



(Photo courtesy of Dr. W.C. Marquardt)

From the development cycle, it's obvious that in temperate climates almost the entire larval population will be present in the stomach during winter since adult egg-laying activity ceases with the advent of the first frost in the fall. For this reason, it had been recommended that a single treatment after the first frost would effectively control bots in the equid. Equids treated like this often still have large numbers of bot larvae in the stomach. Since larval stages of the common bot may remain viable in the egg for a prolonged period of time in the fall and winter months after fly activity has ceased, they provide a constant source of infective larvae for the equid to ingest during this time. For this reason, the old recommendation that treating equids one time soon after the first frost is no longer valid. **For effective bot control in the equid, two treatments are required.** The first treatment for bots should be given in the first worming after the first frost. Then, if you worm at two-month intervals as is often recommended, the next bot treatment should be given four months following the first treatment to achieve complete bot control in the equid.

The only class of drugs available for controlling bots in equids is the avermectins. Organophosphate compounds and piperazine-carbon disulfide complexes, used in the past to control bots, are no longer available in the United States. The avermectins marketed are ivermectin and moxidectin. Ivermectin is marketed under several trade names and moxidectin is marketed as Quest. Both of these drugs control the early larval stage migrating in the tongue and bucal mucosa, as well as larvae in the stomach. Moxidectin previously labeled to control only the second and third larval stage of the common bot, has received FDA approval for the third larval stage of the throat bot.

GETTING THE MOST FROM YOUR CYTOLOGY SUBMISSIONS

Robyn Allison

Nothing is more frustrating than taking the time and energy to submit a sample for cytology only to hear that the sample was "non-diagnostic." Here are a few tips to help prevent this from happening to you.

General Do's and Don'ts:

- DON'T expose cytology slides to formalin fumes. Slides should be mailed in a separate container from specimens in formalin. Formalin has an adverse affect on the staining quality of cytologies, and can result in such poor staining that cells are unidentifiable.

- DON'T expose cytology slides to excessive heat or cold. Once slides are made, they should be air-dried and kept at

room temperature. Don't refrigerate or fix the slides in any way before submission.

- DO take several aspirates of any mass so that multiple slides can be evaluated. This helps reduce sampling error, as not all areas of a single mass contain similar cell types. It also makes it possible for you to stain one slide and evaluate it yourself before submission. Does it look like you aspirated a reasonable sample? Is it just blood? You may decide to try the aspiration again if the sample is of low cellularity. Be sure to send the slide you stained along with the other air-dried, unfixed slides; sometimes that one slide is the only one containing a diagnostic sample!

- DO be gentle when making slides of aspirates, as neoplastic cells (especially lymphocytes) can be extremely fragile and ruptured cells cannot be reliably identified. Generally, just the weight of another slide laid on top of your sample is enough to spread it into a monolayer of cells, then gently pull the slides apart. Remember that samples that are too thick also are very difficult to evaluate, since individual cell characteristics cannot be appreciated.

- DO send blood for a complete blood count along with bone marrow aspirates. Remember to make fresh blood films and send them with the EDTA tube to prevent aging artifacts to leukocytes. If you have already performed a CBC from the same day as the marrow aspirate, please send the results **plus** an unstained blood film. Bone marrow interpretations depend upon the peripheral blood picture for that particular day.

Other Things to Remember

Cells in fluid samples can deteriorate and bacteria can grow very quickly in transit. Again, fresh smears (air-dried and unfixed) sent along **with** the fluid sample can make all the difference. If the fluid looks cloudy, a direct smear can be made (similar to a blood film). If the fluid looks clear, centrifuge a small aliquot as you would for a urine sample, and make a smear from the sediment.

Mast cell granules may stain poorly or not at all with quick stains, so having slides that we can stain with Wright-Giemsa is very important to help us make the diagnosis of mast cell tumor.

Please include as much detailed history and information about from where the aspirate was taken as you possibly can; feel free to call us and give us more information if you need to. Interpretation of the cells we see on cytology depends on this knowledge! For example, granulation tissue can look almost identical to a sarcoma; knowing that the aspirate was from a site of previous injury can help us give you the best possible interpretation. Histories are also very important in the interpretation of bone marrow changes. Remember that we're in this together, so help us to help you!

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Cytology: Submit samples as described above. Fee=$20.

ACUTE PASTEURELLOSIS IN ADULT DAIRY CATTLE—We recently saw an acute outbreak of a virulent *Mannheimia hemolytica* (formerly *Pasteurella hemolytica)* in adult dairy cattle. Sixty out of 1000 cattle became ill and 17 of those died suddenly over a 14-day period with signs of acute respiratory distress. Rapid diagnosis, proper vaccination, and antibiotic treatment controlled the problem.

VETERINARIANS IN THE "LAST GREAT RACE"

Randy Basaraba

The Iditarod Trail Sled Dog Race (affectionately referred to as the "Last Great Race") is an annual sled dog race across the expansive interior and Arctic coast of Alaska. The Iditarod begins the first Saturday of March, with Iditarod 2001 beginning in Anchorage, Alaska, on March 3. Hundreds of volunteers including cooks, dog handlers, pilots, and others are essential to the race. In addition, approximately 30 veterinarians from all over the United States, Canada and Europe take time from their daily routines to ensure the safety and comfort of the hundreds of northern breed sled dogs that will tow sleds and mushers approximately 1,200 miles in as little as 10 days.

Most of the veterinarians on the Iditarod Trail are practitioners who, for the love of the race and the sport of sled dog racing, have taken time (10 days to two weeks) away from family and business to donate their services to the canine athletes. In addition, there are university faculty members and veterinarians representing industry who participate. Many of the veterinarians are members of the ISDVMA (International Sled Dog Veterinary Medical Association), an association specifically for individuals with an interest in the sport of sled dog racing and medicine. The ISDVMA promotes the health and ethical care of racing sled dogs and has hundreds of active members in the United States, Canada, and throughout Europe. The ISDVMA sponsors training seminars for individuals interested in becoming a trail veterinarian, supports research in sled dog physiology, pathology and medicine, and maintains a list of sled dog activities including upcoming races and opportunities for trail veterinarians. The association also maintains a bibliography on past and current published reports of topics pertaining to sled dog medicine. This year's symposia was held October 18-21 in Wisconsin.

Veterinarians participate in the pre-race physical examination and confirmation of animal identification using microchip technology. Prior to the start, teams of three to four veterinarians are distributed along the trail by small airplane to one of the 27 or more checkpoints along the trail. Checkpoints contain provisions for both mushers and dogs that are shipped prior to the start of the race. Each team consists of no more than 16 dogs and total teams can number from 60-80 or more. Checkpoint accommodations range from tents to private homes, schools and community centers. Checkpoints are manned by volunteers including local natives who serve as race officials, dog handlers, and cooks. Veterinarian responsibilities at the checkpoints include observing dogs for lameness or distress as they arrive and performing brief physical exams on all participating dogs before they continue along the trail. Animals that are fatigued or have a physical condition that may require more complex treatment are dropped from the race at checkpoints and are cared for by the veterinary staff and other volunteers until the animal can be transported to its home kennel.

When the majority of teams have progressed through any given checkpoint, the veterinary staff is transported ahead to examine the teams as they progress along the trail. Each musher must maintain a team diary where the physical condition of each dog is documented by the veterinary staff. This system allows for continued monitoring of the physical condition and the effectiveness of treatment for those receiving medication for each individual animal throughout the course of the race.

Being a veterinary staff member on the Iditarod and other sled dog races requires many long, sleepless hours and dedication, but the rewards are numerous. The confines of four walls and the warmth of an indoor examination room are traded for the spectacular scenery of the frozen north country -- wide open spaces where Northern Lights shine and wolves are heard howling in the distance. The meeting of new and different people allows for exposure and understanding of cultures that differ markedly from our existence in suburbia. The greatest reward, however, is the opportunity to work with one of the most impressive endurance athletes of the animal kingdom, the racing sled dog. Working as a team, they pull 300-500 pounds on a sled for 10 or more days in subzero temperatures while burning calories at a rate of 10,000 or more a day. They are the true athletes of the "Last Great Race."

For more information about becoming a member of the ISDVMA and opportunities to become a sled dog race trail veterinarian, visit the ISDVMA website at http://www.isdvma.org.

GET TO KNOW YOUR LAB/Meet Our Virology Staff

Our Virology staff has years of experience, although only a few of our clients have ever had the opportunity to meet them in person. Daily they receive calls from both local and faraway clients who need assistance with result interpretation, sample submission questions, exposure concerns, and more. General questions are typically fielded by our Client Services staff, but they rely heavily upon the Virology staff for more complex problems.

Jane Carman graduated from Colorado State University with a degree in microbiology and has worked with the Diagnostic Laboratory since 1984. Her current job duties include: virus identification; electron microscopy; ELISAs; fluorescent antibody testing; immunohistochemistry; AGIDs; rabies testing; a number of polymerase chain reaction (PCR) tests for IBR, BVD, CDV, CHV, ICH, CPV/FPV, FIV, BLV, EHV, BTV/EHD, OPP/CAE, food animal mycoplasma, and OHV-2, the suspected agent of MCF (malignant catarrhal fever). Jane has had the opportunity to work on a number of research projects over the years and currently is working on MCF in dairy cattle and Bovine Respiratory Coronavirus in dairy calves. Jane also is interested in new test development and is working on new PCR tests for feline coronavirus (FIP) and BRSV, as well as new serology tests for CDV, CPV, FPV, FHV, and FCV by ELISA. Jane enjoys spending time working with undergraduate and graduate students on their independent studies or graduate research.

Anita Schiebel, a Colorado State University graduate with a degree in Microbiology, has been with the Diagnostic

Kathi Wilson, Jane Carman,
Anita Schiebel, Gail Chinnock

Laboratory for 17 years, with the first four years in Bacteriology. Anita spends the majority of her time working on viral serologies, especially serum neutralizations (SN), blue tongue virus isolations, and PCR tests for feline herpesvirus, chlamydia, and MCF (OHV-2).

Kathi Wilson is another long time, dedicated employee who works in the milk culture section, as well as virology. Kathi, a graduate of Colorado State University with a degree in microbiology, started working at the Laboratory in 1994. Kathi is responsible for the set-up and read-out of all milk cultures and also performs the FIP and Ehrlichia IFAs, and BVD Capture ELISA tests.

Gail Chinnock (formerly Miller) is the most recent addition to the Virology Laboratory. Like Jane, Anita and Kathi, she also is a Colorado State University graduate in microbiology (May 1999). Gail started here shortly after graduation and devotes her days to viral serology, primarily canine parvo and distemper vaccination titer determination. She also is responsible for maintaining multiple-cell culture lines used for both research and diagnostic testing. In addition, Gail is working on mycoplasma PCR testing and fills in for other technicians during their absences.

We hope this brief summary of the Virology staff helps you get to know whom to call with questions and to put a face with that friendly voice on the other end of the line!

DIAGNOSTIC APPROACH FOR ORGANOPHOSPHORUS AND CARBAMATE TOXICOSES

Dwayne Hamar and Cathy Bedwell

Organophosphorus (OP) and carbamate insecticides are cholinesterase inhibitors. They non-competitively inhibit the enzyme, rendering it inactive, which results in the accumulation of acetylcholine. OPs tend to bind the enzyme irreversibly, whereas carbamates tend to inhibit the enzyme reversibly. Therefore, the clinical effects of most OPs last longer than do those of carbamates. When OPs are bound to cholinesterase, some may undergo de-alkylation over time, resulting in a more stable bond between the OP and the enzyme. This is referred to as 'aging.' For this reason, cholinesterase activity may remain depressed until new enzyme is synthesized.

The toxicities of individual OPs and carbamates vary greatly within both groups of insecticides. Since carbamates are not covalently bound to cholinesterase, most are not as toxic as OPs. Exceptions to this general rule include carbofuran (Furadan®), aldicarb (Temik®) and carbamate nematocides, which are very toxic. These substances have been used in the past for malicious poisonings.

Clinical signs of toxicoses result from an increased concentration of acetylcholine at the axon terminal neural end plate. These signs include increased salivation, lacrimation, urination, and diarrhea (SLUD). Some OPs produce axonal degeneration, also referred to as delayed OP neurotoxicity.

When OP or carbamate toxicosis is suspected, begin by obtaining a good history, as well as documenting clinical signs. If, after obtaining this information, OP or carbamate toxicity is high on the list, then submit whole blood (for clinic cases) or brain (frozen ASAP) to us for cholinesterase activity determination. If the cholinesterase activity is at least 50% below the normal range, samples should be analyzed for OPs and/or carbamates. When intoxication by ingestion is suspected, stomach contents are the best sample to submit for analysis. Liver may be analyzed, but most OPs and carbamates are relatively labile and have short half-lives. Since insecticides are commonly used in animal's environments, detection of low levels of OPs or carbamates in tissue does not necessarily confirm the cause of death especially in the absence of a good history, clinical signs, and depressed cholinesterase activity. Because of this, testing of samples for the insecticides is not useful when used ALONE.

We determine acetylcholinesterase activity based on whole blood, serum/plasma, and brain samples. Samples submitted for specific OPs and carbamates, or screens are referred to other state diagnostic laboratories.

OP/Carbamate testing, submit samples frozen. Fee=$12.

ANNUAL CONFERENCE WET LABS
Sunday afternoon, January 7, 2001

- Trichomonas Testing—Drs. John Cheney and Wayne Cunningham will review techniques involved in taking samples for Trichomonas testing in bulls.

- Chronic Wasting Disease Testing—Dr. Terry Spraker, Dr. Wayne Cunningham, and Dennis Madden will review techniques for submitting proper specimens for Chronic Wasting Disease testing. For this test to be valid, proper sampling is crucial.

Look for more information about these wet labs from Colorado State University's Annual Conference organizers.

IMMUNOELECTROPHORESIS: AN IMPORTANT TOOL IN THE DETECTION OF MALIGNANT LYMPHOMA, MYELOMA, AND LYMPHOPROLIFERATIVE DISORDERS

Anne Avery

Immunoelectrophoresis (IEP) is a technology used to detect the presence of monoclonal immunoglobulin proteins and to evaluate for immune system disorders. Recent technical improvements have made IEP more accurate and sensitive, but the test often is overlooked by practitioners when faced with a perplexing case that may be a malignant lymphoma or myeloma.

One reason that the test may be underused is the notion that a patient's total globulin levels must be elevated in the presence of a monoclonal gammopathy, such as those seen in cases of multiple myeloma. However, in recent years, we have seen a number of cases involving monoclonal gammopathies in animals with normal serum globulins. One common explanation for this clinical scenario is the presence of an IgA-producing tumor. IgA constitutes only about 5% of the total serum immunoglobulin, so even dramatic elevations in the IgA levels may not substantially increase the total globulins. Early disease involving a myeloma or B-cell lymphoma producing any of the immunoglobulin subclasses also may not increase the total serum globulins, but qualitative evidence of a monoclonal gammopathy still can be seen with IEP. The following cases illustrate these scenarios.

CASE 1—An 8-year-old fox terrier presented to the clinic with an abdominal mass involving her pelvic bone. Her CBC and biochemical panel were essentially normal, including a low normal globulin of 2.5g/dl. Cytology and histology of the mass and associated peritoneal fluid failed to give a definitive diagnosis. Differential diagnoses were malignant lymphoma and multiple myeloma. To distinguish between these and formulate an appropriate treatment plan, serum was taken for IEP. The figure upper right shows the precipitin arc seen when patient serum (lower arc, "p") and control serum (upper arc, "c") are separated on a gel and reacted with an anti-IgG. The features of this patient's precipitin line that suggest monoclonality of IgG are: 1) the arc is shifted to the left when compared to the control, suggesting that the patient's IgG migrated differently from the control and; 2) the precipitan arc is not symmetrical, as would be seen with polyclonal IgG. The features of polyclonality can be seen in the figure lower right, which shows data from another patient without monoclonal gammopathy. Subsequent studies (PCR for lymphoma, immunohistochemistry of an additional biopsy sample) confirmed the presence of an immunoglobulin producing tumor.

Patient with an IgG monoclonal gammopathy (lower arc) compared with a normal control (upper arc)

Patient with no evidence of a monoclonal gammopathy (lower arc) compared with a normal control (upper arc)

Patient or control serum is placed in the small round well and electrophoresed. Antiserum specific for different subclasses of immunoglobulin (in this case IgG, but could include antibodies for IgM and IgA) is placed in the long trough and allowed to precipitate the patient's immunoglobulin. The precipitin line is revealed by staining for protein.

CASE 2—A 9-year-old border collie presented to the clinic for inappetence and occasional shifting leg lameness. Included in his history was a successful treatment with antibiotics for an abscess. His blood work revealed mild anemia (PCV of 30) and slightly elevated globulins (4.1g/dl with a high normal of 3.4g/dl). In order to determine if the increased globulins were the result of the immune response associated with the abscess, or from another process, IEP was performed. As shown next page upper, a monoclonal IgA gammopathy was noted. In this case, the feature most suggestive of a monoclonal gammopathy is the "fingernail clipping" appearance of the precipitin arc (note that the arc from the patient is significantly deeper than that of the control). For comparison, an IEP from an animal with a polyclonal elevation in IgA is shown next page lower. After further evaluation, myeloma was definitively diagnosed in this dog and treatment initiated.

Patient with an IgA monoclonal gammopathy (upper arc) compared with a normal control (lower arc)

Patient with a mild increase in IgA that is polyclonal (upper arc) compared with a normal control

While many cases of B-cell origin neoplasia can be easily diagnosed with cytology or histology, most practitioners have probably embarked on a tumor hunt in a patient with vague clinical signs, and often this work-up can be expensive and invasive. IEP, along with several other assays that we offer (PCR for antigen receptor rearrangements, flow cytometry) can make this search more fruitful and less invasive. A variety of B-cell origin neoplasms can produce a monoclonal gammopathy; myeloma, cutaneous plasmacytoma, B-cell lymphoma, and B-cell leukemia. Examples of situations in which IEP would be an appropriate assay can include a lytic bone lesion, sustained lymphocytosis, chronic diarrhea, and weight loss in a patient in which other causes have been ruled out. With a complete history, we may also be able to suggest other assays that can aid in diagnosis.

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IEP: Submit .5ml serum or plasma. Fee=$26.

LISTERIOSIS: A Continuing Problem

Charles W. Dickie/Rocky Ford Branch Laboratory

Toward the end of April 2000, a feedlot in Colorado was experiencing cattle deaths in a number of pens that were widely separated from each other. Three animals had circled, collapsed, were unable to rise, and died within 24-48 hours after symptoms had appeared. Two additional animals in different pens were down when veterinarians were called. One steer had a temperature of 104°F and the other had a subnormal temperature. Fluids and antibiotics were given, but the animals died. The veterinarians suspected listeriosis or other brain infections because of the circling behavior.

Gross necropsy findings were unremarkable. Fresh brain from each steer, along with serum and blood, as well as fresh liver and lung from one animal, were submitted to us. White blood cell counts for both animals were elevated, but diagnostic panel findings were not definitive. Both brains were ground in nutrient broth using a mortar and pestle and sterile sand. The homogenate was plated onto blood agar and MacConkey's agar and placed into a 10% carbon dioxide atmosphere. Thioglycollate broth also was inoculated, and all cultures were incubated at 37°C. Homogenate was preserved under refrigeration for a week and recultured in like manner, but we were unable to grow *Listeria* from these specimens.

Formalin-fixed brain histologically showed lymphocytic perivascular cuffing and multifocal microabscesses in the brain stem. Listeriosis was diagnosed, and the feedlot animals were placed on a high level of Aureomycin in the water. This treatment was effective and there were no further deaths. The antibiotic was removed after a week.

A few days later, cattle began dying again and two live downers were presented to us. The cattle were unable to rise and the owner expected them to be dead within a few hours, if they followed the pattern previously seen. The steers were euthanized and the tissues taken were processed as before. Histopathology again showed lymphocytic perivascular cuffing and microabscesses in the brain stem. In this case, *Listeria monocytogenes* was cultured and identified.

The cattle were again placed on a high level of Aureomycin. When treatment was stopped after two-weeks, there were no more deaths. It was thought the animals broke with the disease while still ingesting infected silage. The Aureomycin was killing the *Listeria* as the silage was ingested, but when the antibiotic was removed, the disease recurred. By the end of the second treatment period, it was assumed that the infected pocket of silage had been consumed, and therefore, there was no recurrence.

Ten, 700-800 pound cattle were lost in this outbreak. *Listeria* species are widely distributed, and they have been isolated from soil, decaying vegetable matter, silage, sewage, animal feed, fresh and frozen poultry, fresh and processed meats, raw milk, cheese, slaughterhouse waste, and asymptomatic human and animal carriers. *Listeria monocytogenes* has been isolated from 42 species of mammals and 22 species of birds, fish, crustaceans, and insects. Data suggest the organism has successfully evolved to produce virulence factors only when introduced into an animal or human host. Abortions and visceral forms of the disease must be guarded against, as well as the neural form.

LOW-YIELD ANAEROBIC CULTURES

Doreene Hyatt and Robert Jones

We often are asked to do anaerobic cultures on specimens and some of these cultures are of low value because they are almost always negative or the results are of unknown significance when positive. When we get these requests, we do not know if there are specific reasons for the test being requested, or if it is a matter of an error in filling out the forms. Since we have difficulty in contacting everyone who has requested a low-value test, we perform the test, sometimes causing a delay in turnaround time and always costing you and your client more money. This being the case, we thought that it would be useful to remind you of some tests that are considered of low value diagnostically and some indications for requesting an anaerobic culture.

One example of low-value testing is that we were asked to do over 150 anaerobic cultures on urines. From these, only one had any anaerobes isolated and this anaerobe (a *Clostridium* spp.) was in very low numbers. We are suggesting that rather than spend the money on an anaerobic culture, first test for aerobes. If you have tested for aerobes and *Mycoplasma* sp. and had no growth, but there is evidence of an inflammatory reaction in the urinalysis, it may be time to test for anaerobes. We realize that sometimes it is logistically difficult to get a second urine sample and you want to save time by doing everything at once, but this is one test which we consider to be of very low value.

Below are some further examples of low value tests. Although sometimes anaerobes will be isolated from these samples, the value of these isolations is unknown. Most, if not all of these samples, also will contain high numbers of aerobes.

- Anaerobic culture of sites that contain populations of anaerobes (feces, vagina, distal urethra, oral cavity; unless Clostridial enteritis is suspected)
- Anaerobic culture on urine/urinary/genital tract samples (pyometra excepted)
- Anaerobic culture on central nervous system samples (unless an abscess is present)
- Anaerobic culture on eye samples (surface sampling, intraocular infection may justify anaerobic culturing)
- Specimens submitted for anaerobic culture that are NOT in appropriate anaerobic transport devices

We have found common sites or conditions that contain anaerobic bacteria. They are – draining tracts; abscesses; pleural, pericardial and peritoneal effusions; pyometra; osteomyelitis; pulmonary abscesses; and Clostridial abomasitis or enteritis.

Typical clues that you may be dealing with an anaerobic infection are:

- Foul odor to specimen
- Location of infection in proximity to a mucosal surface
- Infections in bite wounds
- Gas in specimen
- Pus or tissue necrosis evident
- Hemorrhage in abomasums or intestine
- Infection unresponsive to aminoglycoside treatment
- Presence of "sulfur granules" in exudate
- Failure of bacteria observed on Gram stain of original exudate to grow aerobically

Aerobic cultures: Submit sample in port-a-cul or sterile container. Fee=$11. Aerobic and Anaerobic cultures: Submit sample in port-a-cul. Fee=$31.

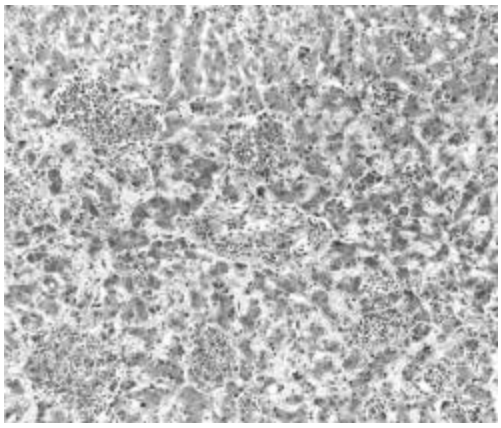
SULFUR INTAKE CALCULATOR ON THE WEB—Due to our dry weather this year, there have been increases in "blind staggers" or polioencephalomalacia associated with high sulfur intake in cattle. On our Web site (http://dxlab.cvmbs.colostate.edu/dlab) is a "sulfur intake calculator" which allows recognition of potentially hazardous conditions by estimating total sulfur intake based laboratory measurement of sulfur content of field samples of water and feed or forage.

EMERGING DISEASE—HEPATOPATHY WITH EXCESSIVE LIVER COPPER IN DALMATIANS

Gary Mason, Cathy Bedwell and Dave Twedt

Canine copper storage disease is defined as a metabolic defect in copper metabolism where copper accumulates in the liver resulting in liver damage that may progress to chronic active hepatitis and a terminal cirrhosis-like architecture. Although copper storage disease is well documented in West Highland White, Bedlington, and Skye Terriers, emerging evidence indicates abnormal copper storage also may be involved in the pathogenesis of liver disease in other canine breeds.[1]

Two published case reports link elevated liver copper with hepatic architectural change and failure in Dalmatians.[2,3] We have identified four similar cases in Dalmatians. Two of these animals succumbed to liver failure characterized by hepatocellular degeneration and necrosis with abundant intracellular Rhodanine positive (copper) pigment, similar to the lesions described in copper storage disease in terriers. Despite the small number of cases identified, abnormal hepatic copper storage with associated liver damage appears to be an emerging disease syndrome in the Dalmatian breed.



Dalmatian liver, centrilobular area: Hepatocellular loss with copper accumulation in hepatocytes and clusters of macrophages.

Biopsy may be pursued in Dalmatians with chronically elevated liver enzymes or used as a screening tool in asymptomatic individuals, much like the current practice in affected terrier breeds. Criteria for case identification include documentation of lesions consistent with abnormal copper storage, subjective visual assessment of copper stores in specially stained sections, and demonstration of elevated liver copper by quantitative analysis.

Either fresh or fixed tissues can be used for quantitative analysis. There are two sampling strategies and either works well. The first is submission of fresh and fixed liver separately. For quantitative analysis, the minimum sample size is one, 1cm long, needle biopsy, but two or even three sections are preferred. Fresh liver is best submitted in an empty (dry) red top tube. When fresh and fixed tissues are submitted separately, copper determination and histopathology are pursued concurrently and there is no uncertainty about the potential for copper loss by leaching into the fixative. An alternate strategy is submission of three, 1cm long, needle biopsies, all fixed in formalin. Following histopathologic examination, remaining liver tissue is melted out of the paraffin block and used for quantitative analysis. This scheme yields acceptable results and ensures that quantitative analysis is performed only on liver tissue and not on sections of diaphragm, lung, connective tissue or skin that are sometimes encountered in needle biopsy samples. Additionally, since all assessments are performed on the same sample, these can be correlated.

Copper chelation therapy appears effective in affected Dalmatians identified early in the disease course. However, identification of additional cases is necessary for an accurate assessment of response to therapy and further understanding of the pathogenesis of this emerging disease syndrome. Dr. Dave Twedt or Dr. Craig Webb at the Colorado State University Veterinary Teaching Hospital (970-221-4535) can address questions regarding clinical aspects of copper storage disease in the canine.

[1]Thornburg LP. A perspective on copper and liver disease in the dog. J Vet Diagn Invest 2000 Mar; 12(2):101-10.
[2]Napier P. Hepatic necrosis with toxic copper levels in a two-year-old Dalmation. Can Vet J 1996 Jan; 37(1):45.
[3]Noaker LJ, Washabau RJ, Detrisac CJ, Heldmann E, Hendrick MJ. Copper associated acute hepatic failure in a dog. J Am Vet Med Assoc 1999 May; 214(10):1502-6.

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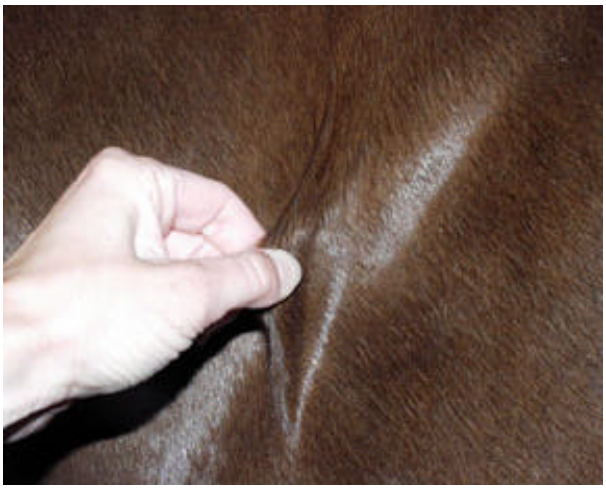
Liver biopsy and quantitative copper analysis: Submit liver as described above. Fee=$32.

HYPERELASTOSIS CUTIS IN HORSES—An Emerging Problem

Pat Schultheiss

Hyperelastosis cutis of horses is a skin disease characterized by excessive stretchiness of the skin and separation of the skin from underlying connective tissue. The skin can be pulled away from the body for a distance of 5cm or more. Seromas form where the skin separates from deeper tissues and the seromas are often the first abnormality noted. The condition affects the topline from the poll to the tail, lateral thorax, and occasionally hindlegs as low as the stifles. The disease is usually recognized in the second year of life. Sometimes, it is noted when the horses are saddled and skin tears under the tack, but some horses tear their skin simply by rolling on the ground. Histologic features are variable and biopsy alone cannot definitively diagnose the condition. Biopsies from several locations of a suspected case are useful, however, particularly in eliminating other causes of skin disease. No treatment is known and most affected horses have been euthanized.



Excessive skin stretching in a horse with hyperelastosis cutis.

This appears to be an emerging problem. More than 20 cases with biopsies and pedigree information have been examined by us since 1997. Faculty in the Diagnostic Laboratory and Veterinary Teaching Hospital have consulted on additional cases. Affected animals are quarter horses from performance horse breeding lines. Both sexes are affected. Pedigree analysis, electron microscopic evaluation, and investigation of biochemical defects in dermal collagen are being conducted.

For more information, or if you would like to share observations of cases you have seen, please contact Dr. Patricia Schultheiss of the Diagnostic Laboratory at 970-491-7376 or Dr. Stephen White, Department of Medicine at the University of California/Davis, at 530-752-5908.

INTERSTITIAL PNEUMONIA IN PUPPIES

Dan Gould

We offer a polymerase chain reaction (PCR) test for canine parvovirus (CPV) types 1 and 2. The well-known CPV-2 causes acute enteric disease of dogs. The effects of canine parvovirus type 1, also known as minute virus of canines (MVC), are less understood. Experimental oronasal exposure of specific pathogen-free puppies with CPV-1 resulted in inapparent to severe disease. Those that developed severe illness had lesions that included bronchitis and interstitial pneumonia with various degrees of lymphadenitis. Enteric signs or lesions were absent in the experimentally infected animals. Bronchial, bronchiolar, and alveolar epithelial cells appeared to be the sites of viral growth and correlated to the pattern of histopathologic changes. In another study of a naturally occurring canine abortion late in gestation, MVC antigen could be demonstrated in fetal tissues by immunofluorescence. The seroprevalence of MVC hemaglutination-inhibiting antibodies appears to be high in adult dog sera from widely separated geographic areas of the United States.

In our necropsy laboratory, we commonly observe puppies that died after a brief course of illness. Some of these pups have histologically demonstrable interstitial pneumonia. A review of our records reveal that in 27 cases of puppy mortality tested with the CPV PCR, 7 (26%) were positive. Since no other pathogens were demonstrated (canine distemper virus, canine herpes virus, heavy cultures of bacteria indicative of septicemia/bacteremia) and enteric lesions were absent, it is assumed CMV is the pathogen involved in these puppy deaths.

CPV PCR: Submit fresh chilled or formalin-fixed tissue. Fee=$23. for PCR, $22. for histopathology.

We have had two cases of strychnine toxicity in dogs in the Kersey area. Please watch for this.

JOINING FORCES

Barb Powers/Wayne Cunningham/ Frank Garry/Cleon Kimberling

The State Veterinarian's Office and Regional Laboratory, and Colorado State University's Veterinary Diagnostic Laboratory, Integrated Livestock Management Program and Cooperative Extension for Veterinary Medicine are joining forces to form a cooperative Colorado Animal Health Center. The center will provide comprehensive support to the agriculture livestock industry, other animal owners, and veterinarians. This cooperative effort will increase efficiency of diagnostic and health surveillance services, and allow us to more rapidly identify and monitor disease outbreaks. Working together, we will be better able to identify animal health issues that need further in-depth research conducted by graduate students in the Integrated Livestock Management Program. This also allows the State Veterinarian's Office and Cooperative Extension to coordinate their outreach education to veterinarians, animal industries, and interested animal owners. A key component of this effort is to educate veterinary students early in their program to provide a more comprehensive understanding of diagnostic and regulatory medicine. We hope more students will then choose these areas of veterinary medicine for their future careers to help meet the national demand for veterinarians in these areas.

This cooperative effort involves the building of a new facility to house these entities and students. The new facility will be located in Fort Collins near the current Diagnostic Laboratory and Veterinary Teaching Hospital. The facility, currently in planning stages, still has to go forward for State approval and funding. In the meantime, we continue to work together on numerous projects and are planning for the future. We are most excited about the potential for this Center. If you have any questions, please contact any of us.

ONYCHOBIOPSY WITHOUT ONYCHECTOMY: Description of a New Biopsy Technique for Canine Claws

Ralf Mueller and Thierry Olivry

The following is a precise of an article describing a new biopsy technique for canine toenails/claws. This technique is important because it avoids toe amputation. The most important differential diagnoses include immune-mediated diseases such as Pemphigus, infections and the idiopathic

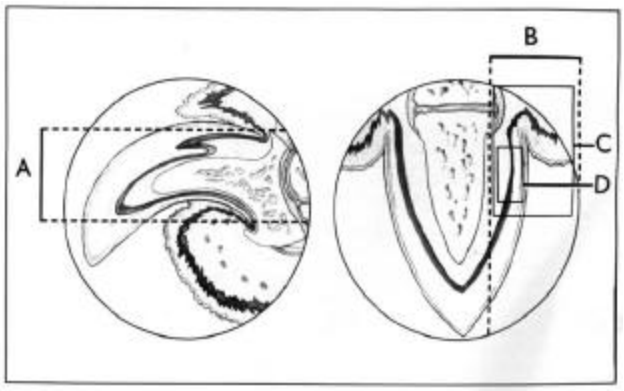
lupus-like toenail disease. Please contact Dr. Sonya Bettenay at 970-491-1281 for further details.

Biopsies of the claw and microscopic examination of the claw matrix epithelium are usually necessary to achieve a specific diagnosis for most onychopathies in the dog. When claw biopsy specimens are required, distal onychectomy has been the method advocated. Distal onychectomy often meets with owner reluctance, necessitates a prolonged surgical procedure, and results in post-operative pain and discomfort for the animal. The technique described here yields appropriate samples of the claw epithelium. This procedure is rapid, elementary, and avoids unnecessary amputation of the distal digits therefore obtaining owner permission for specific diagnostic tests more readily.

Biopsy of the claw matrix, including part of the adjacent bony tissue, is a painful procedure. General anesthesia and, in some cases, post-procedural analgesic therapy is indicated. Dogs biopsied with this technique recovered without problems and showed no signs of lameness or discomfort at suture removal, and at follow-up examinations six-weeks and three-months after the procedure.

Surgical Technique

- General anesthesia is required and should be performed with agents having good analgesic properties. Regional nerve blocks may further reduce the pain during and after the procedure, which is short and in experienced hands should not require more than five minutes per sample obtained.
- If possible, take specimens from affected dew claws to avoid subsequent clinical signs of pain from weightbearing. If dew claws are not present or not affected, sample one or two claws of one rear paw. Transient lameness of the affected leg for one or two days may be seen.
- Clip the hair on the selected distal paw, but surgical preparation is not recommended, as important surface clues may be removed with scrubbing.
- Use a tourniquet to decrease the sometimes significant hemorrhage during the procedure.
- Place the biopsy punch (8mm diameter) horizontally parallel to the axis of the claw 1-2mm distal of the claw fold.
- Take the biopsy specimen by rotating the punch slowly in one direction deep into the tissue. Direct the punch medially initially through the horn of the daw to gain a "purchase" and then through to the bone of the distal phalanx and laterally through normal skin on the lateral aspect of the claw fold.
- Obtain sufficient depth by rotating the biopsy punch 1-2mm into the bone of the distal phalanx (typically 4-5mm).
- Use a scalpel blade (#11) to cut through the base of the sample. Considerable force may be needed in this procedure but the technique will not work if the toenail is normal (the punch will likely break).



Two-dimensional planes. The punch is placed parallel to the claw axis on the medial or lateral edge "D" in this sketch.

- Gently dab the tissue sample on gauze and apply **India ink** to the **haired surface.** Fixation is achieved with 10% buffered formalin.
- Use at least two sutures with nonabsorbable suture material to close the site and achieve ligation of the injured vessels. Place one suture at the proximal edge of the biopsy site and apply tension at closure to ligate the dorsal common digital artery. Pull the skin lateral to the site over the exposed bone and suture to the opposite side with one or two more sutures.
- Bandage the distal limb to protect the paw for two to three days.
- Analgesic therapy is recommended for at least eight -10 hours following the procedure.

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Dermatology: Submit sample in 10% formalin as described. Fee=$50.

CVMA past-president Dr. Larry Mackey looking for divine intervention as Dean Jim Voss prepared to tell another joke.

LABEL YOUR SAMPLES!

Be complete when filling out paperwork! Please help us provide you with quality diagnostics by providing us with complete information. Please, ALWAYS label your samples; we receive hundreds of samples daily and we cannot guess who sent them if they are not labeled.

RESULTS ON-LINE!!

Contact Jay Kammerzell (970-491-1281) for passwork-protected access to all your laboratory results.

WHAT'S INSIDE

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College of Veterinary Medicine
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