

Colorado State University Veterinary Diagnostic Laboratories



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Letter from the Director

Spring brings you our first issue of LabLines for the year 2001. Food Animal disease certainly has been in the news lately with the outbreak of foot and mouth disease in the United Kingdom, and concerns over Transmissible Spongiform Encephalopathies (Chronic Wasting Disease, Scrapie, and Bovine Spongiform Encephalopathies). The increased recognition of the potential impact animal disease can have on our nation's economy reminds us of the importance of the animal disease diagnostic testing and surveillance that we all do daily. The importance of disease surveillance will only increase in the future.

Since the last issue, we had a successful meeting with our External Advisory Committee, who advise us on the quality of our service and help us determine our future directions. New committee members are Drs. Larry Mackey, Todd Towell, Robyn Elmslie, and Michael Gotchey. We greatly appreciate the advice and input of this committee. We were pleased to see many of you at the CSU Annual conference in January. Our new virologist, Dr. Hana VanCampen, has been a tremendous addition to the laboratory and you will find numerous articles written by her in this issue. We continue working with the State Veterinarian's office and the architects on our proposed new combined building—we are looking forward to a new era of cooperative effort in the State of Colorado.

We have completed our 2000 Annual Report of laboratory activities and disease statistics. Please call us if you would like a copy or look it up on our web site. Within this issue of LabLines, you will find a client/user survey that we hope you will fill-out and return to us (or complete it on-line). We will use this information to improve our services to you. We hope you have a wonderful spring and summer, and look forward to seeing you again in the fall. We will be at the Colorado Veterinary Medical Association Annual Conference in September at Steamboat Springs and hope to see many of you there!



Barbara E. Powers

Barbara Powers, DVM/PhD/DACVP

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GETTING THE MOST OUT OF BOVINE SEROLOGY --Hana VanCampen

Our virology/serology section routinely offers the following bovine serology tests:

Serum neutralization(SN)	AGID/ELISA
IBR (aka BHV-1)	Bovine leukosis virus
Bovine herpesvirus-4 (BHV-4)	Blue Tongue
BHV-5	
Type 1 BVDV	
Type 2 BVDV	
BRSV	
PI-3	

History—To get the best value from your bovine serology, the following information is helpful to the diagnosticians:

- Presenting complaint (abortion, respiratory disease, duration of illness)
- Type and age of cattle (beef versus dairy, cow-calf herd versus feedlot, calves versus lactating cows)
- Number of animals in the herd, number ill and number dead
- Vaccinations (brand of vaccine, modified live (MLV) versus killed, age at vaccination)
- Date of last vaccination and product used
- Colostrum intake as serology tests do not distinguish between passive (maternal antibodies) and active immunity induced by infection or vaccination

Sample Selection—Timing and selection of animals to sample vary depending on the type of disease affecting a herd.

- In cases of acute illness, paired serum samples (acute and convalescent) are more likely to be useful information than a single sample. If a virus is involved in the disease, a four-fold increase will be observed. A single SN titer is unlikely to yield definitive information since it does not distinguish between current infection, previous exposure, or vaccination.
- A SN test on a single serum sample only is useful in unvaccinated animals.
- Paired serum samples from an aborting cow rarely will show a change in titers. Usually, cows infected with the BVD and IBR viruses abort weeks to months after infection. Therefore, it may be more useful to compare SN titers from cows with healthy calves to the titers of cows that have aborted.
- Fetal serum samples can be helpful in some cases. If the fetus was infected during the last half of gestation, it may have made antibodies to the infecting virus.

Test Selection—The type of test performed depends on the herd history.

- For example, if type 2 BVDV was isolated from a fetus or calf in the herd, type 2 BVDV SN tests might be indicated. An animal infected with type 2 BVDV generally will have lower antibody titers to type 1 BVDV.

Bovine Serology: Submit 5cc blood/serum. Fee=\$5-6 depending on test.

EXPERIMENTAL STUDIES ON EQUINE SUBCHONDRAL BONE CYSTS

--Bob Norrdin

Subchondral bone cysts are focal radiolucencies seen below the articular cartilage in radiographs of the stifle, fetlock, and other joints in horses. They usually are seen in performance horses and have been associated with trauma, osteochondrosis, and degenerative joint disease. The size and contents of these radiolucent areas vary and their etiology is not always clear.

Recently, Drs. David Frisbie and Chris Kawcak of the Equine Orthopaedic Research Laboratory completed a study of healing in full-thickness cartilage defects (ie., the calcified cartilage layer removed) versus partial-thickness cartilage defects (the calcified cartilage layer left intact) in the medial femoral condyles of 12 horses. The arthroscopically produced defects were “micropicked” to puncture underlying trabecular bone and stimulate healing. Upon evaluation 12 months later, variably-sized subchondral bone cysts were seen. This represented an opportunity to study a collection of bone cysts morphometrically. This project was completed by Karl Hoopes, a member of the Veterinary Class of 2003, who is interested in equine orthopaedics. The objectives were to see if the calcified cartilage layer, although perforated, represented a barrier to cyst formation and/or expansion, and whether the contents of the cyst varied with size. It was found that there were one or more cysts in half of the operated joints whether or not the calcified cartilage had been removed. Cyst size was about the same, although the largest cyst was in the group with full-thickness defects. The bony wall of the cysts was similar in amount of bone and active bone formation. Within the lesions, there was mostly fibrous connective tissue, usually around a central cystic space. Woven bone and fibrocartilage could be found closer to the bony wall. Larger cysts had a greater proportion of cystic space and loose fibrous connective tissue than smaller cysts. In some of these, resorption of bone was seen inside the cyst wall suggesting pressure-induced expansion. The perforated, calcified cartilage layer was not a significant barrier to enlargement. A connection was found between the cyst and the articular surface in three of six cysts from one group and in four of six cysts from the other group. This supports the hypothesis that synovial fluid forced through cartilage defects played a role in development of the cysts. It

has been estimated that synovial fluid pressure is 100 times greater in some joints than marrow sinusoidal pressure. Interestingly, prostaglandin E2 and nitrous oxide, activators of osteoclastic resorption, have been found within equine cyst samples by Dr. Brigitte von Rechenburg of the Equine Orthopaedic Research Laboratory. It's possible that pressure induces resorption and cyst expansion via these mediators.



Subchondral bone cyst that developed under cartilage defect made experimentally. Cysts contained a central space, fibrous connective tissue, and reactive bone formation in the wall. Findings of surface connections indicated that they were due to synovial fluid being forced into marrow spaces.

FOOT AND MOUTH DISEASE

--Hana VanCampen

Foot and mouth disease (FMD) is back in the news with the current epidemic affecting Great Britain, Northern Ireland, the Netherlands, Belgium, and France. A veterinarian examining swine at an abattoir first detected the current FMD epidemic in the United Kingdom. The FMD virus quickly spread to cattle in a neighboring facility. Evidence of FMDV infection has been found at over 100 separate sites. As of March 9, 67,000 cattle, sheep, and pigs have been destroyed in the UK in an effort to control this highly contagious viral disease. The viruses that cause FMD are composed of seven genetically diverse (serotypes) picornaviruses. Serotype specific vaccines for FMDV are used to curtail epidemics in countries with endemic FMDV. Vaccines are part of the current efforts to eradicate FMDV from South America. While vaccination prevents severe disease, it does not prevent infection or excretion of FMDV by vaccinated animals.

The clinical signs of FMD are the appearance of vesicles in the tongue, oral mucosa, interdental space, heels, and coronary band. Vesicles break readily and form ulcers. Affected animals exhibit excessive salivation, anorexia, fever,

and lameness. While mortality is low in FMD, morbidity is very high. FMDV infects cloven-hoofed animals and camels, both domestic and wild. Swine are an excellent source of FMDV as they amplify the virus to high titers. A chronic carrier state occurs in recovered animals in which virus can be demonstrated in the pharyngeal tissues for six-months-to-two-years post-infection.

FMDV is primarily transmitted by contact with saliva and respiratory aerosols from infected animals. Fomites and mechanical transmission by humans also are important sources of contagion. FMDV is sensitive to acidic (pH<6) and alkaline (pH>10) conditions. FMDV survives for extended time in organic matter, including lymph nodes, bone marrow, processed meats, cheeses, and other milk products. The virus also survives for several hours in aerosolized particles under ideal climatic conditions, and airborne virus can be transmitted over distances greater than 1 kilometer.

FMD was eradicated from the United States in 1929. Other FMDV non-endemic countries include the UK, Canada, Greenland, New Zealand, and Australia. Accidental introduction of FMD to Canada in 1951 occurred with the discarding of a sandwich containing FMDV into a feedyard by an emigrant worker from Eastern Europe. FMD is uncontrolled in much of the world. The FMDV isolated in the UK is identical to FMDV that has caused a recent epidemic in Japan and was first isolated in India a decade ago. Other areas that are currently experiencing FMD cases include Iran, Krygyzstan, and Mongolia.

FMD is a reportable disease. In suspect cases of vesicular disease, call the State Veterinarian's Office at 303-239-4161. Samples will be taken and flown to Plum Island for testing. Visit web sites from the State Veterinarian's office (www.ag.state.co.us/animals/reports/fmd1.pdf) and CSU (www.vth.colostate.edu/vth/biosecurity/fmdpolicy.html) for more information.

ABORTION AND DIARRHEA WOES?

--Barb Powers

Now is the time of year we see numerous abortion and diarrhea cases. For your interest, the following two tables delineate what we found in these types of cases in the last fiscal year. Note that for abortions, we often do not establish an etiology, but we can rule-out important infectious causes of abortion. For complete abortion screens, submit either the entire fetus and placenta OR fresh liver, lung, kidney, stomach contents, placenta, and eyeball; and formalin-fixed liver, lung, kidney, heart, brain, thymus, and placenta. Serology screens from the herd also may be useful (see previous article on Bovine Serology). For complete diarrhea screens, submit either a recently dead animal OR fresh tied-off intestine, feces, liver, and lymph node; and formalin-fixed intestine (multiple levels), liver, and lymph node. Please call to discuss sample submissions or results if you have any questions.

ABORTION SCREENS

SPECIES	NUMBER EXAMINED	VIRAL	BACTERIAL	OTHER	UNDETERMINED
Bovine	127	10 (9BVD/1IBR)	32	22 ^a	63(49.6%)
Equine	42	2 (EHV)	7	4 ^b	29(69.0%)
Porcine	3	0	1	1 ^c	1(33.3%)
Ovine	21	0	12	6 ^d	6(28.6%)
Caprine	4	0	1	0	3(75.0%)
TOTAL	197	10	53	33	102(51.8%)

^a2 suspect nitrate toxicity, 13 Neospora, 2 nutritional/toxic, 3 developmental/dystocia, 2 fungal placentitis.

^b4 developmental/dystocia

^c1 congenital defect

^d5 Chlamydial, 1 congenital defect, 3 had both Chlamydia and Campylobacter jejuni

DIARRHEA SCREENS

Numbers are the positive/total tested with (percentages).

Species	Rota	Corona	E. coli	Salmonella	Clostridia	Cryptosporidia	Other	Undetermined
Bovine	17/193 (18.3)	18/193 (19.4)	17/105 (16.3)	23/105 (21.9)	4	33/101 (32.7)	11/105 ^a (10.5)	22/105 (21.0)
Equine	1/16 (6.3)	0/16 (0.0)	0/16 (0.0)	2/16 (12.5)	3	0/16 (0.0)	2/16 ^b (12.5)	10/16 (62.5)
Porcine	0/3 (0.0)	0/3 (0.0)	2/4 (50.0)	0/4 (0.0)	0	0/3 (0.0)	0/4 (0.0)	2/4 (50.0)
Ovine/ Caprine	0/4 (0.0)	0/4 (0.0)	3/4 (75.0)	0/4 (0.0)	1	0/4 (0.0)	2/4 ^c (50.0)	0/4 (0.0)
TOTAL	18/116 (15.5)	18/116 (15.5)	22/129 (17.1)	25/129^d (19.4)	8	33/124 (26.6)	15/129 (11.6)	34/129 (26.4)

^a 1 Johnes, 7 BVD, 2 Strongyles, 1 Coccidia

^b1 Strongyles, 1 Parascaris

^c1 Coccidia, 1 Nematodirus

^d11 *Salmonella typhimurium*

NOTE: Clostridial cultures are done only when there is a suspicion of Clostridial infection, and are not part of the routine diarrhea screen.

Abortion Screen—Submit samples as described above. Fee=\$63 for food animal, \$70 for equine

Diarrhea Screens—Submit samples as described above. Fee=\$63 for food animal, \$70 for equine

CAPRINE HERPESVIRUS-1

--Hana VanCampen

Caprine herpesvirus-1 (CapHV-1), also known as bovine herpesvirus-6, originally was isolated from an outbreak of enteritis and rapid death in kids. Clinical signs reported included fever, dyspnea, abdominal pain, and blood-tinged, watery feces. Affected kids had leukopenia, neutropenia, lymphopenia, and were hypoproteinemic. Kids rapidly became weak and died within three days despite treatment. Gross lesions included ulceration of gastrointestinal tract. Intranuclear inclusions were observed in histopathologic sections of multiple tissues.

CapHV-1 abortions have been reported in North America and Europe. Abortions occur in mid- to late-gestation. Gross lesions in aborted fetuses generally are not observed. Histopathologic examinations reveal multifocal areas of necrosis in livers, lungs, adrenal glands, and spleens. Intranuclear inclusions are found in multiple tissues.

Fetal death and abortion have been experimentally induced in pregnant does inoculated with CapHV-1. Vulvovaginitis and balanoposthitis also have been reported. The prevalence of CapHV-1 infections in goats appears to be widespread. Goats also may be infected with BHV-1 (IBR) and develop similar diseases. In cases of abortions and neonatal kid mortality, send us tissues for virus isolation or PCR for CapHV-1.

Caprine Herpes Virus Testing--Submit tissues on ice. PCR Fee=\$22, Virus Isolation Fee=\$25, OR submit entire carcass for abortion or diarrhea screen, Fee=\$63.

NEWS FROM THE WESTERN SLOPE

--Darrell Schweitzer

In recent weeks, the Grand Junction area has been experiencing what is presumed to be a series of malicious animal poisonings. A number of pets, both dogs and cats, as well as wild birds, have died in a relatively short time in a defined neighborhood and with apparently similar symptoms. The toxicant found was 1080 (sodium fluoroacetate), a very toxic compound that is under restricted use. These cases took several weeks and analysis for many toxicants to resolve thus, a few thoughts regarding cases of suspected poisonings may be in order.

It is important to recognize that suspected poisonings far outnumber actual poisonings. It seems to be human nature to find someone to blame for any and everything possible. Many times, owners of sick animals suspect malicious poisoning as their first thought. Since many suspected poisonings actually are some form of disease, a thorough history and, if possible, physical exam, are important. This often rules-out poisoning.

For those animals in which disease can be ruled-out, and history and clinical signs indicate the possibility of poisoning, carefully selected specimens should be collected. Blood, serum, and urine samples from live animals, as well as any vomitus, should be obtained as soon as possible.

For dead animals, a good history also is important. Find out, for example, what the animal normally eats, what it has been recently fed, if it is ever outside, if it is in a fenced enclosure, or if it runs free. Perform a complete necropsy as soon after death as possible. While many toxicants leave little evidence of gross lesions, any abnormalities should be noted. Are stomach contents consistent with what was learned in the history? Is there evidence of voiding of urine and feces? Lacking gross lesions, a complete set of organ tissues should be collected and placed in formalin. At this point, from history, clinical signs, and post-mortem findings, you should have some idea of what kind of poison may be involved. Consult with the toxicology laboratory to which you plan to submit specimens to ascertain which specimen(s) they prefer for the suspected poison, and how they would like it preserved. As a general rule, collect urine if possible, stomach contents, liver, kidney, fat, ocular fluid, and brain as a minimum.

It is not appropriate to submit samples with the request to "check for poisons." I recall my toxicology professor offering a perfect grade to anyone who could demonstrate any substance which is not toxic at some dose. With that in mind, indiscriminate testing for "poison" is not practical. Along with your specimen submission, include all the information you learned from the history, as well as clinical signs, treatment, and necropsy findings. Any treatment given should especially be noted. Toxicologic examinations, like all things dealing with biologic systems, are not necessarily black and white. If the toxicologist finds evidence of a drug in an "untreated" animal, the test will likely be repeated, resulting in extra expense and time. If a drug is expected, it can be eliminated from further consideration as a toxicant.

While police or sheriff's departments generally are reluctant to become involved with suspected poisonings, it is appropriate to note that when they do the nature of the investigation changes significantly. The suspected poisoning is no longer only a diagnostic case, but a legal one, and new rules will apply. In such cases, be sure to consult with appropriate officials prior to submitting samples to ensure that chain of custody and other legal requirements are met.

Because toxicologic examinations are generally relatively expensive, it makes sense to carefully choose tests that will best delineate those substances which are most likely involved, as indicated by history, clinical signs, and post-mortem findings. Also keep in mind that many tests take time to perform so inform your client that instantaneous answers may not be possible. Finally, we must ensure that animal carcasses are disposed of properly so that environmental contamination, even though only a potential at disposal time, does not occur.

RODENTICIDES

--Dwayne Hamar and Cathy Bedwell

This general article on rodenticides will inform you about some of the different types of rodenticides that your client's animals may be exposed to. Since all rodenticides are sold under various trade names, it is important in assessing rodenticide exposure to read the label of the product to determine the potential rodenticide exposure. Future newsletter articles will focus on the anticoagulant rodenticides and less commonly used rodenticides (with specific trade names) available at least in the Fort Collins area.

Since there are several different types of rodenticides, simply stating that you are dealing with a potential rodenticide toxicity is insufficient information to aid in confirming your diagnosis. Thus, a complete history, including but not limited to clinical signs (if observed), potential exposure to a specific rodenticide (either scientific name or trade name), whether the animal was treated, with what, and the apparent response to therapy are all very pertinent information.

The most common type of rodenticides used are probably the anticoagulants. The first one on the market was warfarin, which has been sold under several trade names. There are several second-generation anticoagulant rodenticides produced mainly because some rodents develop resistance to warfarin. Most of the second-generation compounds are more toxic than warfarin and some are metabolized at a slower rate than warfarin. This means that a therapy regimen which worked well for warfarin will need to be continued for a longer period of time when treating animals exposed to second-generation compounds.

Other rodenticides include cholecalciferol (Vitamin D₃), bromethalin, strychnine, zinc phosphide, ANTU and 1080 (sodium fluoroacetate). Cholecalciferol results in increased mineralization of the soft tissues, especially kidneys. Bromethalin is neurotoxic. Strychnine results in seizures (see LabLines, Vol. 4, No. 2, Spring 2000). Zinc phosphide results in pulmonary edema and acute congestion of internal organs. ANTU is predominately a pulmonary toxicant. The product 1080 causes the formation of fluorocitrate, which inhibits carbohydrate and fat metabolism. It is not marketed as a rodenticide but is licensed for predator control.

Recently, a comprehensive book was published which we recommend for small animal practitioners – "Small Animal Toxicity," editors Peterson and Talcott, WB Saunders Co., copywrite 2001. There are also several websites that contain pertinent information. One that gives information about specific rodenticides, trade names, and EPA registration information may be found at: <http://pmep.cce.cornell.edu/profiles/rodent/index.html>.

GET TO KNOW YOUR LABORATORY/Meet the Word Processing Staff

Word processing is one area of the laboratory that although rarely seen by our clients, is an important "behind the scenes" element. Quick transcription and reporting of histopathology and necropsy cases is essential to our clients, no small feat for this area of the laboratory that averages 500-700 biopsies and histopathology reports, and 40 necropsy reports per week!



Elaine Andersen, JoAnn Paille, and Mary Lindburg

Mary Lindburg has been with the laboratory 11 of the 17 years she has been employed by CSU. Mary is an administrative assistant who spends approximately half her time transcribing necropsy and histopathology reports, as well as faxing and billing cases. The other half of her time is spent processing travel documents, word processing our Annual Report and numerous reports for the Director, and "LabLines."

Elaine Andersen is another long time CSU employee of 17 years who has spent the past five years working at the laboratory as a word processor. Elaine mainly works on transcribing histopathology cases as well as faxing, billing and distribution of case reports. She also coordinates the work for two off-site transcriptionists, Caryn Dorn and Lynn Hawley. Histopathology cases are sent to them for typing and faxing, and received back at our laboratory for the final processing on a daily basis.

JoAnn Paille is a part-time member of our Word Processing team who has been here for almost three years. JoAnn's duties include the transcription of the skin biopsy reports generated by our dermatopathologist, Dr. Sonya Bettenay, as well as the transcription, faxing, and billing of other histopathology cases as time allows.

NITRATES

--Rocky Ford/Charles W. Dickie

Global population increases and the resulting pressure on agriculture to increase production are creating special problems for livestock. A culprit is nitrogen, and livestock poisoning from nitrates is on the rise as the use of these chemicals upset the environmental balance.

In the distant past, lands were worn out when available ammonia, nitrites, and nitrates were depleted. The conversion of atmospheric N₂ (our atmosphere contains 80 percent nitrogen in stable N₂ molecules that are relatively inactive) was too slow to keep up with human demands, and fields were abandoned. But today, we artificially supplement fields with commercially produced fixed nitrogen, adding more of the nutrient than the soil than natural microbial processes are able to. Unfortunately, an estimated 20 percent of the nitrogen we use eventually ends up in lakes, rivers, oceans, and public reservoirs.

With the increase of nitrates in our environment, livestock poisoning from these chemicals also has increased. Acute death from nitrate toxicity may be the alarming thing that we pay the most attention to; but insidious, less dramatic poisoning occurs. At the Rocky Ford Laboratory, we feel that many of the full-term, nonviable calves we see are chronic nitrate problems. Indeed, many ocular fluids from these calves test positive for nitrate. If we can search far enough, often a feed source with high nitrate can be found. Because of the chemical vagaries of nitrate, absolute values for toxic levels are difficult to assess, but changing or eliminating a feed can solve abortion problems. Whenever possible, we recommend an ocular fluid screening test for nitrate as a routine procedure on all fetuses and nonviable-term calves, even if another cause is readily discernible. Sometimes, however, infectious causes of abortion may cause ocular nitrate levels to be artificially elevated.

Nonviable-term calves exposed to nitrates usually are normal in appearance, have a clear trachea, show no infection or changes of internal organs on histology sections, are negative for significant bacteria, and are negative for IBR and BVD. They usually are not autolyzed, and the aqueous ocular fluid tests positive for nitrate. The cows also appear normal. Nitrate problems in livestock probably will increase as the poison continues to accumulate.

FOAL IMMUNOGLOBULIN DETERMINATION

--Doreene Hyatt and Cindy Hirota

Addressing concerns brought to us by equine clinicians at Colorado State University, we have been testing foal sera for immunoglobulin concentrations using various commercially available test kits. Since the discontinuation of the CITE® test by IDEXX, the company has been marketing a SNAP® Foal kit for determination of equine IgG. Another test kit, FOALCHEK®, also has been used in our equine barn.

Our clinicians were concerned about consistency between the tests and we have been analyzing each test against radial immunodiffusion.

SNAP® Foal tests are based on ELISA (enzyme-linked immunoassay) technology and come complete with the reagents and supplies necessary to run the test. Basically, a loopful of sera (OR 2 loopfuls of whole blood) is added to a dilution bottle. One drop of the mixture is dropped on the sample spot. A bottle of conjugate is poured into the well of the snap device. When color appears in the activate circle, the kit is "snapped" (pushed down) which breaks open a reagent capsule. This reagent diffuses across the paper and the test is read after seven minutes. The sample reaction is compared to two standards present on the filter and reported as less than 400, 400-800, or greater than 800mg/dL.

The FOALCHEK® test kit is sold by Centaur, Inc., and is based on latex agglutination. The kit comes complete with the reagents and supplies necessary to run the test. Depending on which end of the concentration spectrum you want to test, there are two procedures. If you are interested in lower concentrations, 5 microliters of sera (or 10ml of whole blood) are added to a diluent vial. After five minutes and thorough mixing, two drops of the mixture are added to a ceramic ring, three drops are added to the second ring, and four drops are added to the third ceramic ring of the glass slide. Two drops of latex are added to each well and mixed with the provided mixing stick. The slide is rotated for 15 seconds and read for the number of rings that contain agglutination. There are no positive controls included for the test kit.

Radial immunodiffusion (RID) is the gold standard of immunoglobulin determination. We use a kit that is manufactured and distributed by VMRD. This test requires an overnight incubation to allow the immunoglobulins to diffuse through the agar that contains an antibody to equine IgG. A ring of the precipitates is visible in the gel at the distance from the well where the optimal proportion of the antigen (equine IgG) to the antibody (anti-equine IgG) is reached. The diameter of the precipitating ring formed by the sample is compared with the diameters obtained with known standards.

Like many other equine groups and diagnostic laboratories, we have found a low correlation between test kits or within types of tests. At this time, while studies are progressing to compare the tests, we are recommending that if there is a questionable result on the SNAP® Foal test, a follow-up be done using radial immunodiffusion. Preliminary results of our comparisons indicate that SNAP® Foal test results between 400 and 800mg/dl are not consistently correlated with the RID. Recent product literature by the manufacturer of SNAP® Foal indicates that the company is improving the test because of customer concerns and we have seen improvement in the comparisons since these changes have occurred. As with any test, the clinical signs of the animal must be used to determine if treatment is required.

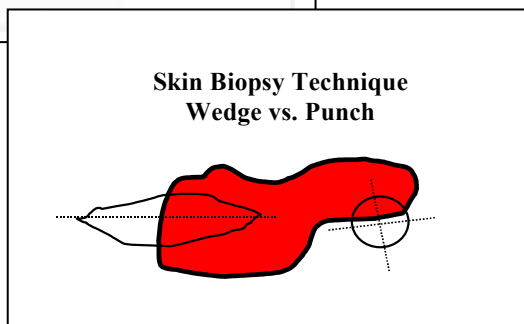
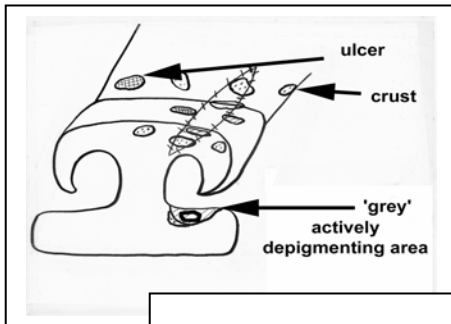
RID for equine IgG—Submit 1ml serum. Fee=\$12.

CANINE NASAL DERMATITIS—How Do We Get More From Our Biopsies

--Sonya Bettenay

Canine nasal dermatitis is a common disease. On a clinical basis, it is frequently diagnosed as “discoid lupus erythematosus” (DLE). DLE is one of the most common cutaneous immune-mediated skin disorders in the dog. It can be confused both clinically and histopathologically with a recently recognized entity – mucocutaneous pyoderma. The characteristic histopathology findings of DLE are well-documented. Yet, there are many cases which fail to provide the diagnostic histopathology, but present with compatible clinical signs and do respond to immunomodulatory therapy. Can we obtain a more diagnostic biopsy by selecting different lesions? Which sites should we choose? Should we choose different clinical lesions to aid in the diagnosis of pemphigus? To further confuse the issue, there frequently is an overlap, with many cases of discoid lupus showing a partial clinical response when trial-treated with a course of systemic antibiotics. Should one pre-treat with antibiotics to obtain a cleaner biopsy field? We know the answers to some of these questions, and are studying others.

Selecting a Biopsy Site—The client pays for the biopsy and they expect a diagnosis from the first attempt! We can try our professional best to accommodate this but it is not always possible, even when one samples multiple, well-chosen sections exhibiting a variety of clinical lesions. A biopsy section is like a frame taken from a roll of movie film. It reveals only what is present at the time of the biopsy sample. Disease is a dynamic process and the clues as to the cause of the disease simply may not be present at that point in time. However, it is possible to provide as many snapshots as we can and use supportive if not absolutely diagnostic results.



A punch must contain 100% of the same tissue (normal or abnormal) to avoid that the wrong part is 'cut in'. All tissue looks gray after formalin fixing.

When selecting a biopsy site for noses, remember that the area with the most pathologic activity (the part we wish to biopsy) is in the epidermis. Pemphigus pustules are in the epidermis (with acantholytic cells sometimes in the crusts), and lupus and mucocutaneous pyoderma changes are in the epidermis and very superficial dermis. Because of this, we do not want to biopsy ulcers where the epidermis and diagnostic lesions are lost. Choose the actively depigmenting or 'gray' areas as well as any superficial crusts (not those deep enough to be covering an ulcer). It also may be helpful to biopsy some haired skin directly behind the planum, even if only mild erythema or scaling is present there. Remember that what you submit will be sliced in half by the histopathology technician. A wedge will be sliced lengthwise, and a punch directly in two in any orientation. It is imperative with punches that they contain 100% uniform lesion. Particularly with nose biopsies, where we are trying to differentiate pemphigus, remember to submit crusts and advise the technician to “please cut in crusts.” Wrapping the biopsy sample in lens paper prior to placing it in formalin will help ensure that the technician finds the crust intact.

Diagnostic Laboratory Nasal Dermatitis Study—To try to evaluate some of the answers to the above questions and to obtain a better understanding of how we should interpret these biopsies, we currently are undertaking both a review of previous biopsies and a new prospective study. If you have contributed a nasal biopsy sample in the past 12 months, you may receive a questionnaire in the mail. Anybody interested in helping us with a new case by submitting a sample and keeping a 12-month follow-up, please contact Dr. Bettenay at 970-491-1281. The outline of the study is as follows:

Protocol summary: This is a multi-centered study. All animals will have clinical symptoms compatible with canine cutaneous lupus erythematosus. All will be biopsied according to a set protocol. All will be subsequently treated with tetracycline/niacinamide, and then prednisone, if the first treatment fails to produce satisfactory clinical results. Within this protocol there will be two subgroups. One will be pre-treated (prior to the biopsy) with systemic antibiotics and the other will have no pre-treatment but will receive the same antibiotic regime after the biopsy. They will then be examined (and a special examination form completed) at two weeks, five weeks, and eight weeks after the biopsy, and then every three months for a 12-month period.

All biopsies will be fixed initially in glutaraldehyde for 24 hours and then moved to formalin for shipment.

Dermatohistopathology: Submit biopsies in formalin. Fee=\$50.00 plus postage or courier fee.

DIAGNOSTIC TESTS FOR CAPRINE ARTHRITIS-ENCEPHALITIS AND OVINE PROGRESSIVE PNEUMONIA

--Hana VanCampen

The Viruses—Caprine arthritis-encephalitis (CAE) is a group of diseases caused by caprine arthritis-encephalitis virus (CAEV). Diseases include encephalitis in kids and arthritis in adult goats. Encephalitis usually presents as a progressive paralysis. Signs of arthritis begin with swollen joints and pain on movement. The arthritis may progress until the goat is unable to move the affected joints. Other diseases caused by CAEV include mastitis or “hard bag,” pneumonia, and wasting. Ovine progressive pneumonia virus (OPPV), a close relative of CAEV, causes similar diseases in sheep. Sheep most commonly develop wasting and chronic pneumonia, but encephalitis and mastitis also occur. Arthritis is less common in this species than in goats. Experimentally, OPPV infects kids causing arthritis and pneumonia, and CAEV infects and causes pneumonia in lambs.

CAEV and OPPV belong to the lentivirus genus within the retrovirus family. An important feature of retrovirus replication is that the virus’s genetic material becomes integrated into the DNA of the infected cell. As a consequence, animals infected with retroviruses are permanently infected. “Lenti” means slow and refers to the length of time from infection to the appearance of clinical signs. Most CAEV/OPPV-infected animals do not develop signs of disease. Observable disease develops in approximately one-third of infected goats, and some symptoms (eg., arthritis) may take years to develop.

Immune Response—Infected goats or sheep develop antibodies that specifically bind to the CAEV or OPPV. Although specific, this immune response does not clear the virus infection and the animal remains persistently infected with CAEV/OPPV. Individual animals may be infected for several months before antibodies can be detected. Antibodies to CAEV/OPPV are transferred to kids/lambs via colostrum, but these antibodies do not protect the kids/lambs from infection. Rather, they are an indication that the kids/lambs have been exposed to and are likely to be infected with these viruses.

Transmission—The primary means by which goats are infected with CAEV is through the ingestion of virus in the colostrum of infected does. Intrauterine transmission (from the infected doe to her fetuses) of CAEV occurs but the frequency is unknown. There is good evidence that CAEV also is transmitted by direct contact with infected goats. This mode of transmission is suspected to occur through ingestion of virus in saliva and feces-contaminated feed or water, or by inhalation of aerosolized virus. CAEV also is present in blood, and can be transferred through blood contamination of needles or syringes. Tattooing equipment also is a potential means by which virus can be transmitted. Similarly, OPPV is

transmitted primarily from ewe to lamb via colostrum, and from sheep to sheep via the respiratory route. Transmission in utero and in feces-contaminated feed have been reported.

Diagnostic Tests Available—CAEV and OPPV infections can be detected in two ways. The first is by demonstrating the presence of virus-specific antibodies in serum. There are two serologic tests for CAEV and OPPV – the agar gel immunodiffusion (AGID) and ELISA-based tests. We perform the AGID test daily and it takes 48 hours to complete. There are a number of different ELISA-based techniques that detect antibodies to CAEV/OPPV; however, we do not offer these currently.

We do perform a polymerase chain reaction (PCR) test for CAEV and OPPV. The PCR detects the virus’ genetic material in white blood cells in a sample of whole blood. The PCR test is set-up once a week and takes 3-to-5 days to complete. None of the diagnostic tests described differentiate between CAEV and OPPV.

Samples Required—The AGID and ELISA tests are performed using serum. A minimum of 5cc of blood should be collected in red-topped blood collection tubes. The PCR test requires 10cc of whole blood collected in EDTA (purple-topped) tubes. Blood and serum should be refrigerated but not frozen, packed well to prevent breakage, and sent to us by overnight mail service.

Additional Tips for Diagnostic Tests—Plan ahead if animals need to be tested prior to sale, show, or export. Call us in advance to obtain the test schedule, or if a large number of samples need to be tested. Clearly identify each goat’s sample by name or ID number.

Test Result Interpretation—The majority of CAEV/OPPV infected animals will be positive by the serologic tests and positive on the PCR test. Some infected goats are antibody negative and PCR positive. These animals are infected with CAEV/OPPV, but have not yet seroconverted. Individual infected animals have been shown to intermittently become seronegative.

Some infected animals will be antibody positive and PCR negative. These animals may harbor CAEV/OPPV infected cells in lymph nodes, bone marrow, or neural tissue. The virus in these organs stimulates the immune system to make antibodies. At the time of sampling, however, there may not be any CAEV/OPPV present in the blood sample, and therefore, the PCR test will be negative. If antibody or PCR positive animals are identified, the herd should be considered to be infected.

Uninfected animals will be antibody negative and PCR negative. If negative animals are found in an infected herd, they will need to be re-tested over several months after separation from positive animals. Re-testing is needed because of the sometimes lengthy interval between infection and the appearance of antibodies or virus in blood samples.

Prevention and Control—Currently, there is no cure or vaccine for CAE or OPP. Preventing infection should be undertaken on a herd/flock basis. If a herd/flock is negative for CAEV/OPPV, any new purchases should be tested prior to their introduction. This method is not foolproof as infected animals may take several months to seroconvert, and some infected animals will be PCR negative.

Positive-test animals should be removed from the herd/flock as they are a source of infection for other animals. The most economical testing procedure is to use serology to detect CAEV/OPPV positive animals for culling. Any remaining negative animals should be tested using the PCR test. Repeated testing is necessary due to the chronic and covert nature of CAEV/OPPV infections. Testing also is useful in surveillance in CAEV/OPPV negative herds.

If, for some reason, it is desirable to retain the offspring of positive dams, the kids/lambs should be removed from the dam at birth and fed colostrums from uninfected does/ewes, or colostrums that have been pasteurized to inactivate these viruses. CAEV/OPPV infections also can be transmitted from animal-to-animal by means other than colostrum. Because of this, test-negative animals should be well separated from any positive animals. Wire fencing is not adequate separation. Milking utensils, feed tubs, needles, etc. used in positive goats or sheep should not be used in negative groups without disinfection or sterilization.

CAE/OPP testing: AGID—Submit 5cc blood/serum, Fee=\$5.00; PCR—Submit 10cc whole blood in EDTA tubes, Fee=\$22.

A RARE BACTERIOLOGY CASE

—Doreene Hyatt and Terry Spraker

A 1-year-old Nubian goat was brought to us with a history of a wound (eight-to-nine months prior) that had developed an untreatable abscess. Upon necropsy, the male goat was found to be in very poor body condition. A large, walled-off abscess (10-14cm) was present on the affected limb and a portion of the abscess had undergone bony metaplasia. Multiple abscesses also were found throughout the lung, thoracic cavity, liver, and within several bones of the ribs. The lymph nodes around these abscesses were enlarged. Histopathology from the liver and lung revealed large abscesses that had a fibrous wall that was heavily infiltrated with neutrophils, macrophages, lymphocytes, and plasma cells. The center of the abscess was filled with cellular debris and inflammatory cells. There was no evidence of multinucleated macrophages in any of the areas.

Think that you know what the problem was without culture results? When the bacteriology results were finalized, the lung and liver both had high numbers of . . . no, not Corynebacterium pseudotuberculosis, but Rhodococcus equi.

These results were a surprise because Rhodococcus equi most commonly causes diseases in horses, particularly foals from 1-to-6 months of age. In foals, it causes a suppurative bronchopneumonia with large abscesses in the lung. In the Nubian goat, the most likely cause of emaciation and death was that the wound that occurred approximately eight-to-nine months prior was infected with Rhodococcus equi. The bacterial infection became septicemic and caused multiple abscesses throughout the body. So, if you see abscesses in caprines, it could be something other than what you think!

COMBINATION DRUGS PREVENT MORE THAN JUST HEARTWORMS

—John Cheney

In a recent article from Veterinary Practice News, David Congation states, “With the continued emergence of combination drugs, the entire heartworm prevention market is being redefined and re-evaluated by consumers and practitioners alike.” If your clients have not already heard of these products, they soon will. The pharmaceutical companies are developing multi-million dollar advertising campaigns to inform your clients of these new drugs.

These new combination products give protection against heartworms. Some of these products also will control internal intestinal parasites such as the large roundworms (Ascarids) and hookworms, and will work against external parasites such as fleas, ticks, and mites. In using these products, it is important to know the range of parasites the drugs will control.

The first of these combination products to come out on the market was Sentinel™ produced by Novartis. Sentinel™ is a combination of interceptor (milbemycin oxime) and lufenuron. Milbemycin oxime eliminates the tissue stage of heartworm larvae and the adult stage of hookworms, large roundworms, and whipworms. Lufenuron, the other active ingredient of Sentinel™, does not kill adult fleas, but breaks the flea life cycle by inhibiting egg development.

The next of these combination products is Heartgard Plus™ marketed by Merial. Heartgard Plus™ is a combination of ivermectin and pyrantel pamoate. Ivermectin controls the tissue stage of the heartworm and pyrantel pamoate controls ascarids and hookworms. Heartgard Plus™ by itself does not control external parasites. It can be used in combination with Merial’s Frontline™ (fipronil) to kill adult fleas, flea eggs, and ticks.

The newest of these products is Revolution™ (selamectin), marketed by Pfizer. Revolution™ kills adult fleas and prevents flea eggs from hatching. It controls ear mites in puppies and kittens. Revolution™ also can be used to treat and control sarcoptic mange in puppies, to treat kittens for hookworms and ascarids, and to prevent heartworm disease in dogs and cats.

GETTING SAMPLES TO THE LABORATORY

We frequently get calls on how to submit samples to our laboratory. We have a User's Guide that tells you what, how, and where to submit, as well as testing schedules and fees. Call us if you need a copy or look it up on our Web site.

A quick review of how to get samples to us follows.

- **FedEx courier service**—Fee depends on package weight and location.
- **Direct Ship/Overnight Mail**—Fee depends on package weight and location.
- **Regular Mail/UPS Service**—Fee depends on package weight. For biopsy samples, we have pre-filled mailers

that we send to you free-of-charge and have a \$1.50 return fee. Due to unsolvable problems with fluid leakage as we tested the environmentally-friendly, non-toxic fixative, we are returning to formalin containers that do not leak!

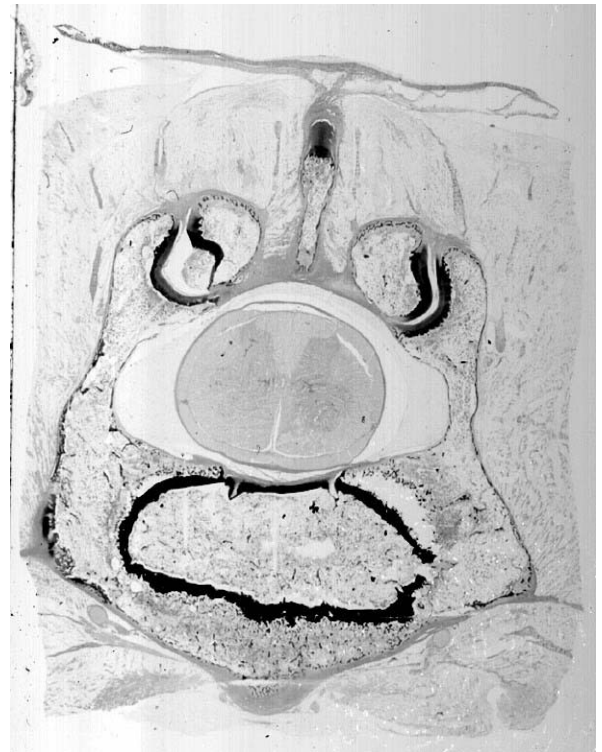
- **Local Courier Service**—For the Fort Collins, Loveland, and Greeley area, fee is \$7.50 per pick-up, more for distant locations. We contract with Golden Age Courier Service. Call us to arrange a pick-up.
- **Large Animals for Necropsy**—Independent contract driver Daniel Rogers will truck carcasses for necropsy, fee depends on location. Contact number is 970-590-2299.
- **Drop-Off**—Come by any time to visit us and drop your samples off directly to the office area. We love to see your smiling faces. After-hours drop-off areas with refrigerators are also available.

RABIES!!

Last year in Colorado, we had a positive case of rabies in a bat. The public health office also had some positive bat cases. Any bat acting strangely or coming in contact with humans should be checked for rabies. As you may have heard, a case of rabies in a young fox from the Canon City area was also recently reported by Public Health officials.

ON-LINE RESULTS

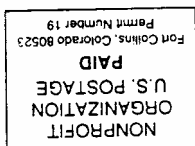
Currently, we have over 200 on-line users. You may obtain results on all your cases at any time using this system. This is password-protected so that only you can access your results. For those of you utilizing the Fort Collins laboratory for EIA testing, we are scanning the completed Coggins form that you can print out. The system requires NETSCAPE 4.0 or greater, IE 4.0 or greater, and Adobe Acrobat Reader. Call Jay Kammerzell at 970-491-1281 to obtain a password, or Email him at jay_kammerzell@colostate.edu.



One pathologist to the other: “Ok Maynard, what is your diagnosis? Is it a Pluto or a Brutus; or perhaps one of those very rare clowns from across the tracks.” In reality, this is a microscopic cross-section of vertebral column including spinal cord, from a young animal with osteopenia due to vitamin D deficiency.

WHAT'S INSIDE THIS ISSUE:

- Bovine Serology
- Equine Subchondral Bone Cysts
- Foot and Mouth Disease
- Abortion and Diarrhea Results
- Caprine Herpesvirus
- Poison Cases From the Western Slope
- Rodenticides
- Nitrates
- Foal Immunoglobulin Testing
- Nasal Dermatitis in Dogs
- CAE and OPP
- Rare Bacteriology Case
- Drugs to Treat Heartworms



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