Colorado State University Veterinary Diagnostic Laboratories

Volume 3, Number 2 Spring 1999

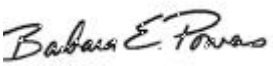
Letter from the Director

With the arrival of spring, this edition of LabLines provides some seasonal topics as well as topics applicable to any time of year. We hope you enjoy the articles and find them informative. We have started a new section of the newsletter entitled "Get To Know Your Lab," where we will introduce you to the people who serve you.

Since our fall issue six months ago, we have continued to expand and improve our diagnostic service. In January, we met with our External Advisory Committee, comprised of producers and practitioners spanning all animal industries and interests. Members of this group donate their time and expertise to advise us on our performance and guide us in our future endeavors. Their input is most valuable and this meeting provided us with many ideas for future improvements. Feel free to contact them or us directly if you wish to provide input into our laboratory function and development. The names of the Advisory Committee members and the industries they represent are listed inside.

Inside, please note information on the new computer-based access to our laboratory. Be assured this is a password-protected system so only you have access to your results. Please send us comments about this, or any other topic, you would like to see addressed in LabLines.

Enjoy your spring and summer, and we'll see you in the fall!



Barb Powers, DVM/PhD

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DIAGNOSTIC LABORATORY
ADVISORY COMMITTEE MEMBERS

Mr. Gene Schoonveld/CDOW	Dr. Joan Bowen/Small Ruminants
Mr. Norm Brown/Equine	Dr. Marv Hamann/Cattle
Dr. Greg Goodell/Dairy	Mr. Ed Hansen/Cattle
Dr. Lenny Jonas/Small Anim	Dr. Tony Knight/Clin Sci
Dr. Del Miles/Cattle	Dr. Mike Miller/CDOW
Dr. Wendell Nelson/VTH Director	Dr. Ken Neuens/Equine
Dr.Jerome Geiger/Swine	Dr. Brian Wooming/Poultry
Dr. Pete Walker/CDOW	Dr. Steve Wheeler/Small Anim
Dr. Wayne Cunningham/St Vet Office	Dr. Mary Gray/CSU Coop Ext

ABORTION DIAGNOSIS
Darrell Schweitzer/Western Slope

Spring renewal is upon us, with new arrivals in the animal kingdom making their appearance daily. Unfortunately, as we know, not all arrive alive, so a few reminders for submission of samples from aborted fetuses are in order. I generally handle animals that die shortly after a term birth without ever gaining their feet the same as I do premature animals, so these remarks apply to those animals as well.

I frequently get telephone calls inquiring about submission of a single sample to confirm a particular diagnosis. While a single sample may sometimes prove adequate, if that particular sample is not diagnostic for the suspected condition, there is often no way to further investigate the cause. Frequently, determination of the cause of abortion is difficult at best. Therefore, we prefer a generalized screen approach to abortion diagnosis, so we can look for many possible causes in all cases.

Submitting a whole intact fetus, with placenta if possible, to the laboratory is most advantageous. Many times, clients are willing to drive to the laboratory to deliver the fetus personally, but if not, the fetus can be shipped. Ship the fetus packed in ice, but not frozen, by overnight delivery. A frozen carcass not packed with ice will often thaw during transport, and a formerly dry shipping container may be leaking by the time it arrives at the laboratory. So, be sure to package the fetus adequately to contain all liquid. This will prevent hard feelings or outright refusal to deliver by the carrier's delivery personnel.

If sending the whole fetus is not practical, perform a thorough necropsy. Collect and package samples as aseptically as possible. (NOTE: An OB sleeve tied off into segments with a different tissue between knots does NOT qualify as aseptic.) For all species, collect the following minimum tissues and fluids for submission:

- in formalin--heart, lung, liver, kidney, spleen, thymus, brain, and placenta.
- fresh (keep cold, not frozen)--lung, liver, kidney, abomasal or stomach contents, and placenta.
- deep frozen (if cannot submit soon)--lung, liver, kidney and ocular fluid.

Tissue size in the latter two categories should be of culture size, ie., at least 2-3 cm^2. Placenta and abomasal fluid is especially important in sheep abortions. Include specimens from any other abnormal findings as well. If you have access to the dam, this would be a good time to draw an acute blood sample for possible serology.

A good history is important to include with your laboratory submission. Request an abortion screen, plus any other specific tests you may want to have performed. Please inform your client

that diagnosis probably will not be instantaneous. While we report positive findings as soon as they are available, it may be necessary to wait until all results are in before drawing a final conclusion, which could take up to four weeks. While this may be frustrating to a producer suffering losses, it is still a worthwhile pursuit. Abortion outbreaks are often too late to correct at the time of abortion and the value of a diagnosis lies in prevention the next season.

Abortion screen: Submit fetus or tissue as described above. Fee=$60.

Causes of Abortion 1997-1998 (Fort Collins Laboratory Only)					
Species	Number Examined	Viral	Bacterial	Other	Undetermined
Bovine	108	9 (8BVD/1IBR)	21	22[a]	56 (51.9%)
Equine	42	1 (EHV)	7	4[b]	30 (71.4%)
Ovine	22	0	9	7[c]	6 (27.3%)
Porcine	7	2 (PRRS)	2	1[d]	2 (28.6%)

a/7suspect nitrate toxicity, 6 Neospora, 3 nutritional/toxic, 6 developmental/dystocia

b/4 developmental/dystocia , c/6 Chlamydial, 1 developmental, d/1 fungal

CAVEATS OF SEROLOGIC TESTING (reprinted from the Annual CVMA Meeting, 1998) Jim Collins

Quantitative serodiagnosis requires the measurement of the level of specific antibody, and results are reported as "titers." Quantitative results are generally reported as either significant (**that level of antibody which indicates exposure and possible protection**) or insignificant **(the animal is susceptible**). If paired serum samples are submitted, the results may be reported as a **seroconversion,** which is evidence of an infection during the time the samples were collected.

- •**Seroconversion**-Represents a 4-fold or greater increase (or, rarely, decrease) in titer between the acute and convalescent samples. The 4-fold or greater rule is derived from the observation that serologic tests have a ±50% variability.
- •A **seroconversion** also is defined as the change from "negative" to "positive."
- •A test for seroconversion must be performed in the same laboratory, and, ideally, in the same test, on the same day, by the same person.
- •Knowing the timing of sample collection relative to the length of infection in the animal is important for an accurate interpretation of the titers.
- •There is great individual animal variability in "titer" responses. In a herd outbreak, several animals should be sampled in order to arrive at an accurate interpretation of the titers.
- •Vaccination causes a rise in titer if administered shortly before or during the time period between the paired serum samples.
- •Routine vaccination may make use of **diagnostic** serology difficult or impossible, because the background level of antibody already is high and a further 4-fold rise is unlikely.
- •With some infectious agents and in some situations, background titers may be high from field exposure of wild-type viruses. Infectious agents may be circulating sub-clinically. Background titers found in these situations are also of limited usefulness.

Serology testing: Submit 1ml serum. Fee=Variable/test.

TOXOPLASMOSIS

Mike Lappin

Toxoplasma gondii IgM and IgG testing by ELISA is available for both dog and cat serum. The presence of *T. gondii* IgM in serum is consistent with recent or active infection. *Toxoplasma gondii* IgG can be present in serum for years and so rising titers must be demonstrated to document recent or active infection. If we are alerted, convalescent and acute sera are run at no additional charge on the same ELISA plate to avoid interassay variation. Demonstration of *T. gondii* specific antibody production in aqueous humor or cerebrospinal fluid correlates better with ocular or central nervous system disease than serum alone. Submit both serum and aqueous humor or serum and cerebrospinal fluid for this assessment. An antibody production index (Ctc value) of >1 is consistent with antibody production by the eye or central nervous system. Polymerase chain reaction (PCR) for detection of *T. gondii* in aqueous humor or cerebrospinal fluid is currently available on request at no charge. Assays for detection of IgG antibodies against *Borrelia burgdorferi* or *Rickettsia rickettsii* also are available. Presence of IgG antibodies against these organisms document exposure but not necessarily recent or clinical infection.

The following table shows the number of positive titers for the listed disease agents and species. The numbers listed are positive/total tested and (percentages).

Species	Toxo IgG	Toxo IgM	Toxo CSF IgM	Rickitt-sia IgG	Borrelia (IgG)
Canine	166/963 (17.2)	16/961 (1.7)	0/117 (0.0)	29/68 (42.6)	37/55 (67.3)
Feline	820/3504 (23.4)	41/3475 (1.2)	4/74 (5.4)	NA	NA

Toxoplasmosis testing: Submit 1/2ml serum. Fee=$8.50.

GET TO KNOW YOUR LAB/Meet Our Sample Receiving, Processing Staff and Phone Receptionist

Clients dropping off laboratory submissions in person will most likely be greeted by Janet Tamarak. Janet works on samples arriving through the mail as well as those dropped off from our local clients. They are checked for appropriateness depending on the test(s) requested, split as needed and routed to the correct area(s) of the laboratory. Janet also is involved in the entry of cases into our database and has recently moved into this position. In the past, you may have been assisted by her over the phone. Clients phoning the laboratory are often helped by Jennifer Swenson. Jennifer has worked in our main phone area for two years and also inputs cases in the database, and bills cases.

Behind the scenes are Judi Tuffield and Lee DeBuse. Judi processes the large volume of histopathology samples arriving each day in the mailers provided by our laboratory. She also prepares biopsy specimens by trimming them into cassettes to be processed into slides. Lee works with cases arriving via Federal Express and assists our pathologists with necropsy and research projects, as well as cutting tissues.

LOST IN THE MAIL?

Yes, this does occur and it distresses us as much as it distresses you and your client. Once we had a sample arrive six-months after it was sent! If you have not received results when expected, please call us and we will do all we can to help find the missing sample.

TO FELLOW PATHOLOGISTS--Ever want a test done on your histopathology slide for an infectious agent? We have the following tests available by PCR using histologic sections--*Salmonella* spp., *Chlamydia psittaci* (avian, bovine, feline, ovine), *Mycoplasma* spp. (all, speciated), *Ehrlichia canis*, *Actinobacillus pleuropneumonia*, Bovine Herpesvirus 1, 4, and 5, Bovine Leukemia Virus, Equine Herpes virus 1 and 4, Feline and Canine Herpesviruses, Malignant Catarrhal Fever (Ovine herpesvirus 2), and Ovine/Caprine Lentiviruses (OPP/CAE). Submit 2 parafinized, unstained sections of tissue with your request. Multiple PCRs can be performed on the same submission. Additional tests available soon--BVD, Canine Distemper Virus, Pseudorabies Virus, Influenza Virus. Fee: $22, $23, or $28 per PCR test, depending on agent.

CSU BULK TANK MILK CULTURE PROGRAM
Jane Carman/Jim Collins

The Bulk Tank (BT) Milk Culture Program started approximately 6-1/2 years ago at our laboratory in Fort Collins. Five local dairy producers enrolled in October 1992 and there are currently 85 Front Range producers, averaging just under 1,800 bulk tanks cultured per year. Individual quarter, composite, or bulk tank samples submitted for milk and/or mycoplasma cultures now total more than 17,000 cultures per year.

The BT program screens monthly samples for the presence of bacteria that can be grouped into the following two categories; contagious mastitis pathogens and environmental bacterial contaminants. Initially, tanks were screened for the contagious pathogens *Streptococcus agalactiae (Strep ag.)*, *Staphylococcus aureus*, and *Mycoplasma* spp. Later, counts for total coliforms, environmental Streptococci, Coag.-negative Staphylococci, *A. pyogenes*, and *Pseudomonas aeruginosa* were included. Other saprophytic organisms found to be the cause of high preliminary incubation (PI) counts are also reported when significant numbers (>100 cfu/ml.) are found. These organisms are *Pseudomonas* spp., *Bacillus* spp., and yeast. Information regarding goal level, sources (skin, teat, feces, etc.) and likelihood of the organism to cause clinical mastitis and/or high PI counts also are included within the report. A 12-month rolling calendar has been added recently to assist producers in tracking the changes in counts found within their bulk tanks, since the actual count of these organisms is less important than a sudden rise or recent introduction of a new mastitis pathogen.

Prevalence of each organism is shown below for the 1995-1998 BT Program:

Organisms	1995/% n=307	1996/% n=1457	1997/% n=1542	1998/% n=1774
S. agalactiae	7	9	5	2
S. aureus	52	44	43	40
Mycoplasma spp.	<1	2	4	3
Coliforms	84	80	89	86
Environ Strep	97	98	98	94
Coag-neg Staph	98	97	98	94
Ps. aeruginosa	NA	5	7	5
Pseudomonas spp.	NA	20	16	20
A. pyogenes	NA	2	6	7
Bacillus spp.	NA	<1	<1	<1
Yeast	NA	<1	<1	<1

Prevalence of each organism for individual/composite cultures for 1998:

Organism	1998 (%) n=10,400
S. agalactiae	<1
S. aureus	28
Mycoplasma spp.	5
Coliforms	65
Environ Strep	17
Coag-neg Staph	4
Ps. aeruginosa	<1
Pseudomonas spp.	<1
A. pyogenes	1
Bacillus spp.	<1
Other**	<1

*Multiple isolates within samples results in >100% total.

**May include the following: Yeast/mold/fungi, *Corynebacterium* spp., *Pastuerella haemolytica*, *P. multocida*, *Pasteurella* spp., *Nocardia* spp., *Mycobacterium* spp. (fast growing environmental), and *Prototheca* spp.

Producers considering starting on the Bulk Tank Culture Program should contact their Dairy Farmers of America (DFA) field office. Samples are collected at the Thornton office and sent to us once a month. Additional bulk tank testing or follow-up individual cow cultures should be sent directly to us.

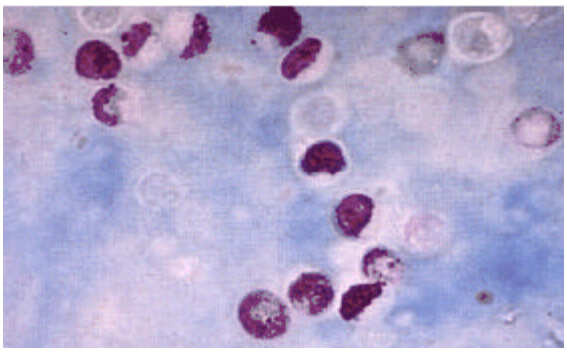
Several recent projects we have completed, or are on-going, may also be of interest to producers. This includes three graduate student projects in the Integrated Livestock Management Program. Dr. Gregory Goodell recently completed work on the study of milk electroconductivity in cows with subclinical mastitis. Dr. John Wenz is completing work on a severity classification of cows with acute coliform mastitis and the differentiation of bacteremic and non-bacteremic cows with acute coliform mastitis. Dr. Heather Hirst just began her graduate studies working with Dr. Page Dinsmore to develop a set of observations and tests that can be used by the dairy producer on a systematic basis to discover the cause of high bulk tank bacterial levels of environmental origin. Anyone currently enrolled on the Bulk Tank Culture Program is eligible for this project and, if interested, should contact Dr. Dinsmore at (970) 221-4535.

***CRYPTOSPORIDIUM*-"WHERE DOES IT COME FROM?"**

John Cheney

Cryptosporidium is a single-celled protozoan parasite that has been identified from a wide variety of hosts, including both wild and domestic animals, birds, reptiles, fish, and humans. The life cycle of *Cryptosporidium* is similar to that of other coccidian parasites, such as *Eimeria*. *Cryptosporidia* was first identified in 1907 in the gastric glands of mice. Until 1975, there were only 15 reports describing *Cryptosporidia* in eight species of animals, and only five of these reports were associated with illness. However, since the late 1970s and early 1980s, there have been numerous reports published regarding the organism. Many of the cases reported were associated with human infections and HIV-positive individuals. As a result, during this period our perception of cryptosporidiosis was transformed from that of a rare and largely asymptomatic infection, to that of an important cause of enterocolitis and diarrhea in both people and animals.

Three species of *Cryptosporidia* are recognized-*C. parvum* in mammals, *C. muris* in mice, and *C. baileys* in birds. *Cryptosporidia* is now known to cause diarrhea in several mammalian species, particularly in young animals including calves, lambs, foals, piglets, kittens, and also in people. In addition, *Cryptosporidia* is known to cause diarrhea in chickens and turkeys. The range of wild animal hosts from which *Cryptosporidia* has been isolated includes foxes, coyotes, deer, beaver, muskrats, squirrels, snakes, fish, rabbits, and mice.



***Cryptosporidia* with acid-fast stain.**

At one time, *Cryptosporidia* was thought to be very host-specific. However, recent experiments have shown that isolates of *C. parvum* will infect several different host species. For example, isolates from calves, humans, deer, goats, and lambs will cross-infect and cause diarrhea in dogs, cats, and piglets.

In recent years, increased interest in regulating animal agriculture has been due in part to a heightened awareness of potential environmental contamination and the spread of zoonotic diseases (those diseases transmitted between animals and people). *Cryptosporidia* is one of the organisms of greatest concern because of its zoonotic potential. *Cryptosporidia* is one of the most common organisms isolated from young calves with diarrhea. Without careful attention to proper sanitation, it is not uncommon for individuals handling these calves to become infected also.

In 1993, an outbreak of *Cryptosporidiosis* in humans, involving more than 403,000 individuals, received widespread media coverage. Initially, the source of the infection was considered to be dairy cattle via dairy pen runoff into Lake Michigan. This water is treated and used for human consumption. However, it was proven that the subspecies of cryptosporidium isolated from this human outbreak does not infect cattle. Media reports implicating the dairy as the source of the infection proved to be both incorrect and unwarranted. Examples such as this should serve as a caution against increased governmental regulations that are not founded on sound science.

In many parts of the country, states and regional districts also are under increasing pressure to reduce the risk of *Cryptosporidia* outbreaks. For example, in California, trail riding and horse operations are faced with increased governmental regulations. In some areas, all horses have been banned from public trails. In a recent California survey, 1,148 horses were tested for fecal shedding of *Cryptosporidia* organisms. Only 12 horses, 1%, were found to be positive for low-level shedding of the organism. In another study conducted by us in conjunction with the Department of Animal Sciences, 300 horses using trails along the Colorado Front Range were tested for cryptosporidia. Only one horse, 0.33%, was found to be shedding the organism.

It's extremely important for livestock producers, veterinarians, and those involved in monitoring and investigating *Cryptosporidia* cases and/or outbreaks to remember that most all domestic and wild animals can harbor and shed the organism. For this reason, it's very difficult to incriminate any one host as the source of an outbreak *Cryptosporidiosis*. Livestock owners, as well as veterinarians, must be aware of the potential zoonotic aspects of the disease and do what they can to reduce the spread of the disease organism.

Cryptosporidia testing: Submit fresh feces. Fee=$9 ($15 for ELISA).

DO YOUR CATTLE NEED DEWORMING?--This summer and fall we will be testing cattle and sheep for internal parasites. This will be done on a herd basis and at a reduced cost. Call us at 970/491-1281 for details regarding sample submission and cost.

NEW *SALMONELLA* PCR TEST

Claudia Gentry-Weeks/Mike Jessen

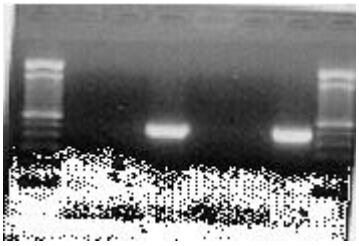
Identification of *Salmonella* sp. from fecal specimens takes five to seven days using standard culture methods. It's often necessary to quickly diagnose animals shedding *Salmonella* sp. in order to implement biosecurity procedures to avoid spreading the infection within a herd of animals. We have developed a polymerase chain reaction (PCR) test for rapid identification of *Salmonella* sp. directly from fecal cultures. The PCR-based method amplifies a DNA sequence spanning a portion of two invasin genes of *Salmonella* sp. The advantage to this test over conventional culture methods is it can provide preliminary results within 24 hours. If a sample arrives before 5PM on one day, the result will be available by 5PM the next day. The disadvantage is that the PCR assay does not provide information on the serogroup, serotype, and antimicrobial susceptibility of an isolate. Therefore, the PCR test will be offered in conjunction with conventional fecal culture to provide optimum results. Preliminary results of the PCR test will be reported to the client by 5PM on the day following submission.

We have examined the *Salmonella* sp. PCR test for 400 fecal specimens. The sensitivity and specificity of the test (the number of tests that correlate with the results of culture method) is 88.9% and 98.8%, respectively, as compared to conventional fecal culture. It should be pointed out that during evaluation of the 400 fecal specimens, only 18 isolates were cultured positive and 16 of the those were PCR positive. The low number of culture-positive isolates correlates to the lower sensitivity (88.9%) of the PCR assay compared with culture results since sensitivity is defined as - [True positive-false negative(not detected by the PCR method)/True positives by culture]. We anticipate the sensitivity of the PCR assay will rise as more culture-positive samples are detected.

Salmonella PCR with culture and sensitivity: Submit 3-5gm feces in a sterile, screw-capped, leakproof container, packaged with a coldpack. Fee=$41.

Ladder/+ Sample/+Control/Ladder

PCR Gel of Salmonella.



(Each end contains a 100bp ladder, one bright spot is a positive sample and the other bright spot is the positive control.)

SCREENING FOR AND TYPING BOVINE VIRUS DIARRHEA (BVD)

Jim Collins

We now use a direct virus-capture ELISA for the detection of BVD. The test, available in kit form from Syracuse Bioanalytical, Inc., detects BVD virus in blood of persistently infected (PI) cattle. These PI animals have virus titers ranging from 10^3 to 10^6 infectious particles per ml of serum. It is

this high amount of virus in serum that enables the ELISA to perform well. In independent evaluations, the ELISA detected 100% of PI cattle, with a specificity of 99.5%. Cattle that have a transient BVD infection are detected only rarely, and if so, the virus remains in the animal's blood at these levels only a few days.

We offer a VI-ELISA that has been a testing standard for several years. This is a more sensitive test because when VI (virus isolation) is added in, small amounts of infectious virus are amplified before the ELISA detection methods are applied. This is an excellent method for screening as well, but one expects to pick up more animals that are transiently infected.

The PI animal is **the most important** source of infectious virus. Such animals not only carry BVD in high levels in the blood, but they also shed the virus in nasal and oral secretions, urine and feces, and the virus is readily transmitted to other cattle by contact with these fluids. A PI heifer or cow will give birth to a PI calf, creating additional animal reservoirs of virus. Spread of the virus to a pregnant cow carries the risk of trans-placental transmission to the fetus, also giving rise to a PI calf. Trans-placental transmission of BVD to the fetus has been observed in vaccinated cows. PI bulls have been shown to produce PI calves, by transmission of BVD at breeding to non-PI cows.

Identification of PI animals in a herd warrants further investigation. Are there higher than usual numbers of open heifers or cows? Are some of this year's calves doing poorly? Do you have other infectious disease problems at an increased level? BVD may contribute to all of these conditions. Perhaps you have suspicions of underlying BVD infection given some of these observations. Most of the screening for BVD we perform is for herds with various non-specific health problems that continue for an abnormally long period of time or do not resolve. Vaccines may or may not be routinely used. The most common tip-off that BVD may be causing herd problems is the recovery of the virus from an aborted fetus. The virus may not have necessarily caused the fetal death, but the finding that the virus was there indicates that it is (or was) circulating in the herd.

With the new capture ELISA targeting the PI animals, we can improve herds by testing and removal of carriers-the ones we need to find! We are able to offer this test at the lowest price ever for BVD screening.

We now perform typing of BVD isolates by polymerase chain reaction (PCR) (types 1 and 2). Typing helps in establishing the antigenic type of BVD involved in an infection and could help select a relevant BVD vaccination program to use. The following table gives a breakdown of the biotypes (cytopathic and non-cytopathic) and the genotypes (1 or 2) and their distribution among BVD disease syndromes, from specimens submitted to us.

In our Colorado area, we see more of type 2 (60%) than type 1 (40%). Type 2 is more prevalent in cases of abortion, respiratory tract infection, and in PI cows. In initial descriptions of BVD type 2 outbreaks, type 2 was found to be associated with a severe, generalized, hemorrhagic syndrome. In the last few years, it has become clear that BVD type 2 can be the cause in virtually all of the BVD-related disease syndromes (just like type 1), and that the hemorrhagic syndrome is not common. As to biotypes, the non-cytopathic types are much more prevalent, and are associated with persistent infections-and a risk of mucosal disease-as has been well-established by many studies.

Distribution of BVD Biotypes and Genotypes
Number of Isolates in Virus Category

	Cytopathic		**Non-Cytopathic**	
	Type 1	**Type 2**	**Type 1**	**Type 2**
Mucosal disease	1	1	4	1
Persistent infection	0	0	11	21
Diarrhea	3	2	9	2
Respiratory disease	1	0	8	14
Abortion	0	2	5	15
Unknown	0	0	1	3
TOTAL	5	5	39	58

From these studies and others, we conclude that the comparable prevalence of both BVD genotypes indicates that vaccines should include both type 1 and 2 strains. These genotypes correspond with segregating antigenic virus types. Cross-protection by vaccination with only type 1 (the most common vaccine) may not give the same level of protection as a vaccine with a type 2 strain.

BVD Virus Capture ELISA: Submit 1ml serum. Fee=$5 for 1-10 samples; $4 for 11-50 samples. BVD Typing: Fee=$28..

THOUGHTS ON SNAKEWOOD POISONING

Charles Dickie/Rocky Ford

Gutierrezia sarothrae, also known as broomweed, snakewood, or broom snakewood, covers a large area of the southwest. It is a native perennial, warm-season plant, reproducing by seed and root systems. The plant has a woody, resinous nature and can grow up to 18 inches high. Small, yellow to gray flowers grow at the tips of the branches. It is considered an evergreen shrub in New Mexico, Arizona, and southeastern Colorado, and is a common poisonous plant largely due to saponins during leaf formation.

Most losses occur as abortions in cattle and sheep. Frequently, acute death does occur in southeastern Colorado during and shortly after spring snowstorms. The plant is considered somewhat unpalatable but will usually protrude above the snow, making it easily available to livestock. Being wet seems to make it more palatable, and hunger and shortness of pasture also contribute to the amount consumed. The plant can cause different degrees of toxicity, related to variations in growing conditions.

Broom snakewood/*Gutierrezia sarothrae*

Signs of poisoning begin after appreciable amounts of the plant have been consumed for several days. Anorexia, listlessness, arched back, drooping head, and icterus are noticeable. Practitioners will usually submit blood to be checked for leptospirosis. This test will be negative; but creatinine, GGT, AST, and BUN usually are elevated. These tests reflect acute toxic tubular nephrosis and toxic hepatitis. More specifically, histopathology of tissues reveal hemorrhages into the nephrons and extensive hydropic hepatic degeneration. Aborted fetuses are essentially without lesions.

An interesting case occurred shortly after a spring snowstorm. A practitioner brought in icteric tissues from a dead cow and requested a leptospirosis check. Grossly, the liver was yellow and swollen, and the kidneys showed some petechia. Silver stains were negative for leptospirosis and hepatic hydropic degeneration was prominent. Blood from icteric live cows showed elevated GGT, AST, creatinine and BUN levels. The owner did not seem willing to accept a broomweed poisoning diagnosis, claiming there was no broomweed present on his range. A site visitation was arranged, and a tour was given and indeed, no broomweed was evident. After returning to the ranch house, I went east into some small canyons and draws where cattle might seek shelter from a storm; there I found extensive stands of broomweed.

Snakewood poisoning is very real and is usually readily accepted as a diagnosis. We have added GGT and creatinine to our standard bovine abortion profile in the hope of obtaining possible data on this plant's involvement in cattle abortions in southeastern Colorado.

DIARRHEA SCREENS

Gary Mason/Barb Powers

The onset of spring brings unsettled weather, crowded birthing grounds, and the arrival of many young animals with limited immunologic experience and defenses. At this time of year, diagnostic laboratories see an increased number of accessions requesting diagnosis of diarrheal disease. We

offer a diarrhea screen designed to detect and identify the agents responsible for this common disease presentation. The list of agents that cause diarrhea with significant frequency is known, leading to a predetermined investigation strategy. However, the differential diagnosis list is periodically reviewed and the approach to detection strategies updated when new technological applications become available. Of course, proper sample submission greatly increases the odds of diagnosis.

Preferred samples include sections of fixed and fresh intestine, including cecal contents and fecal material, as well as other organs you choose to submit, based on gross post-mortem findings or suspicions about the disease presentation that you observe. Ideally, multiple sections of both fresh and fixed intestine are submitted. Submission of multiple sections for histology allows for a better morphologic assessment, both in terms of disease process and time course. Often, the presence and severity of architectural changes is remarkably different in different segments of the intestine. The intestinal tract has a somewhat stereotypical response to injury and some agents produce little or no microscopic architectural alterations.

Additionally, the intestinal tract is prone to rapid autolysis; so sample collection as soon after death as possible greatly aids in structural assessment of the gut. These features necessitate laboratory-based detection methods as an aid in diagnosis. Because attempts to detect multiple agents are pursued in diarrhea screens, submission of multiple loops of bowel, tied off, and submitted within individually labeled whirlpaks provide the most useful and convenient sample for diagnostic laboratory personnel. Samples should be double bagged, shipped in leak proof packages, and kept cool with ice packs. Multiple etiologic agents sometimes are detected in young animals from herd settings and selection of the best animal to sample for diagnosis is important, particularly in on-going disease outbreaks. In this setting, an animal which has suffered a long clinical course is typically less useful for diagnosis than one displaying more acute symptoms and not treated. Antibiotic treatment, of course, can interfere with our ability to detect a bacterial agent. Although the decision to euthanatize a living animal for submission is not an argument most practitioners like to make-- or clients like to receive--there is a time, often following failure of empirical therapy, when this approach presents the best opportunity to gather useful information.

The following table illustrates a breakdown of enteric pathogens we detected for each listed animal species in the 97/98 fiscal year. The numbers are positives/total tested and (percentages). We do not routinely test for Clostridia as part of the "screen" unless signs or histopathologic features are suggestive.

CAUSES OF DIARRHEA 1997-1998 (Fort Collins Laboratory Only)

Species	Rota	Corona	E. coli	Salmo-nella	Clostridia	Crypto	Coccidia	Other	Undeter-mined
Bovine	29/149 (19.5)	11/149 (7.4)	40/177 (22.6)	38/177 (21.5)	24	65/171 (38.0)	8/171 (4.7)	6/177[a] (3.4)	36/177 (20.3)
Equine	2/27 (7.4)	0/27 (0.0)	3/35 (8.6)	1/35 (2.9)	8	0/30 (0.0)	0/30 (0.0)	4/35[b] (11.4)	21/35 (60.0)
Porcine	11/41 (26.8)	2/41 (4.9)	9/41 (22.0)	6/41 (14.6)	2	0/5 (0.0)	1/5 (20.0)	2/41[c] (4.9)	21/41 (51.2)
Ovine/ Caprine	0/7 (0.0)	0/7 (0.0)	1/8 (12.5)	0/8 (0.0)	2	1/7 (14.3)	1/7 (14.3)	0/8 (0.0)	3/8 (37.5)
TOTAL	42/224 (18.8)	13/224 (5.8)	53/261 (20.3)	45/261[d] (17.2)	36	66/213 (31.0)	10/213 (4.7)	12/261 (4.6)	81/261 (31.0)

a/2 BVD, 2 Giardia, 1 Strongyles, 1 Johne's disease

b/4 Strongyles

c/2 Proliferative ileitis

d/23 Salmonella were typhimurium, 3 DT104

Diarrhea Screen: Submit whole animal or tissues as described above. Fee=$60.

WE WELCOME SKIN BIOPSIES FROM HORSES

Patricia Schultheiss/Diane Bevier

When I was introduced to some owners and trainers at a horse show, I told them that I was interested in skin diseases in horses. (I usually do not tell new acquaintances about necropsy service.) One of the responses was very interesting, "Oh good, whenever there is a skin problem people assume it is ringworm and, of course, it rarely is." Today's veterinarians can provide much better diagnostic and therapeutic services for skin diseases in horses, and a skin biopsy is an important tool in providing quality service. In equine practice, the use of skin biopsies is growing. Knowledge of equine dermatopathology is expanding as pathologists and clinicians work together. We are fortunate to have clinicians interested in equine dermatology who are happy to work with pathologists to provide quality service to practitioners. There are times when the skin biopsies do not lead to a specific disease diagnosis but the biopsy findings can help guide the practitioner to certain general categories of disease, prompt additional diagnostic efforts, or suggest therapies that are likely to be useful. Biopsies can help rule-out certain conditions (including ringworm!). Our clients who regularly perform skin biopsies find them very useful and feel they can get a lot of diagnostic information for a relatively low cost.

The major classes of skin diseases include alopecia and haircoat abnormalities, nodules, crusting dermatitis, and scratching. In alopecic conditions, take biopsies from the center of a lesion, its border, and a nearby normal area. With nodular lesions, the entire mass or a wedge biopsy can be submitted. Crusted lesions usually reflect different time points in the development of disease so multiple areas should be sampled. Try to identify the newest lesions. If the crust material separated from the underlying tissue, include it with the specimen and make a note on the history form that it is free in the formalin jar. Pruritic horses also have lesions of varying age so try to sample primary lesions, crusted lesions, and traumatized areas. When performing skin biopsies, be sure to leave the surface intact and not scrub the surface.

Dermatopathology requires teamwork with the clinician. Be sure to include history with the samples. Important points to include are duration, rate of development of lesions, distribution pattern, history of pain or pruritus, general health of the patient, any previous skin problems, and any skin or general health problems in other horses. We look forward to receiving your skin biopsy submissions!

Dermatohistopathology: Submit biopsies in formalin. Fee=$22 plus postage, Fed Ex, or courier charges.

FELINE CONJUNCTIVITIS testing adds on *Chlamydia* and *Mycoplasma* PCR tests--For those submitting conjunctival specimens from cats for Feline herpesvirus PCR testing, you may now request PCR tests for *Chlamydia* and/or *Mycoplasma* on the same specimen. Over the last year, we have developed and evaluated the *Chlamydia* PCR and found it to be highly satisfactory. *Chlamydia* has a lower prevalence than herpesvirus, and clinical signs usually include a more serious exudate. The role of *Mycoplasma* is not well-defined in feline conjunctivitis. Both PCR tests detect a wide variety of *Chlamydial* agents and *Mycoplasma* spp., so watch for more information about using these PCRs on infections of other species of animals. Fee=$23 per PCR test.

MONITORING SERUM BROMIDE

Dwayne Hamar/Cathy Bedwell

Potassium bromide is used alone or in conjunction with other anticonvulsant drugs, especially phenobarbital, for controlling seizures in dogs and occasionally cats. It's important to regularly monitor the serum bromide concentration to be certain therapeutic levels are maintained and not exceeded. Bromide and chloride are both halide ions, therefore, when bromide is given to an animal, the circulating level of chloride decreases slightly. However, if serum electrolytes of an animal receiving bromide therapy are measured by standard clinical pathology techniques, the chloride will appear higher than normal. This is because bromide reacts similarly in the analytic analysis resulting in apparently higher chloride. In fact, if chloride appears to be high on standard clinical chemistry analysis, bromide toxicity should be considered. In these cases, the calculated anion gap will be low.

Bromide determined with the ion specific bromide electrode suffers from chloride interference and will result in a slightly higher bromide level. Chloride does not interfere with bromide determined by ion high performance liquid chromatography (HPLC). We have been analyzing serum for bromide by ion HPLC using conductivity detection for several years. The therapeutic range for serum bromide is 670-2000ppm. After initiation of bromide therapy, serum concentrations of bromide should be determined at four weeks and then again at about four months. The four-week sample is important to detect potential toxicity, however, steady-state concentration is not obtained until about four months after initiation of bromide therapy.

Increased dietary chloride increases the excretion rate of bromide, whereas renal insufficiency results in decreased bromide excretion. Even without the above problems, individual variations in the half-life of bromide have been reported. Thus, serum bromide levels should be monitored regularly.

Bromide test: Submit at least 1ml of serum. Fee=$12.

SWINE CORNER/MYCOPLASMAL PNEUMONIA

Barb Powers

Mycoplasmal pneumonia, also termed enzootic pneumonia, is usually a mild respiratory disease of swine that is both common and widespread. The disease usually affects swine over eight weeks of age and causes persistent coughing, decreased feed efficiency, and decreased weight gain. Carrier swine are common. Pigs also are predisposed to infection with other respiratory pathogens that may cause more severe disease. The reduced feed efficiency makes this a very costly, economically important disease. It's difficult to maintain a Mycoplasma-free herd status.

Pig lung with anteroventral pneumonia, typical for Mycoplasmosis

The etiologic agent, Mycoplasma hyopneumonia, is very difficult to isolate and grow in the laboratory. The gross and histologic features of affected pig lungs, however, are suggestive. Lungs are affected in the anteroventral portions, are atelectatic and red or gray in color. Histologically, there is a chronic pneumonia with prominent lymphoid hyperplasia around airways and arterioles. To confirm the cause of pneumonia, we now have a polymerase chain reaction (PCR) test that can detect Mycoplasma using formalin-fixed, paraffin-embedded tissue sections.

Mycoplasma pneumonia testing: Submit lung in formalin. Fee=$22 for histopathology plus $23 for PCR.

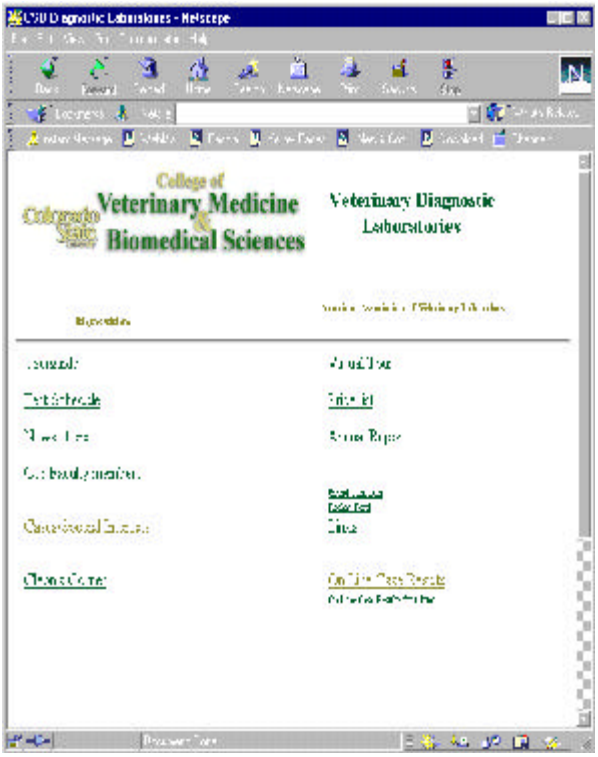
POINT YOUR WEB BROWSER TO COLORADO STATE UNIVERSITY

Jay Kammerzell

We are pleased to make your individual test results available to you on-line! Using a new product called Cold Fusion from Allaire Corporation, we can give you real time access to the results of your cases. You can search for results for your most current submission or as far back as 1994.

The system is password protected and users can search the results **ONLY** of the cases they have submitted. The information is maintained in an Informix database with web access going to a replicated database (a simultaneous exact copy on a separate server) for added security.

This service has been recently upgraded to a new server for faster access and data retrieval. Cold Fusion allows the reports requested via the web browser to come from a variety of sources - our main Informix database, the FoxPro database used by our Biomic antibiotic susceptibility system, text files of final reports of biopsies and necropsies, and other local databases used by a variety of laboratory equipment. The reports are "clickable" options from your lists of cases.



HOMEPAGE

To get access, please contact Jay Kammerzell, the Laboratory's Business Manager, at 970/491-1281 or Email jkammerz@vth.colostate.edu.

We also have added new items to the Diagnostic Laboratory Home Page. New selections include sheep information from our Extension Veterinarian Dr. Cleon Kimberling and information on feline diseases and tests we offer. That address is http://www.cvmbs.colostate.edu/dlab.

Help Us Help You!

- •Please fill out submission forms completely and legibly, including animal information, a brief history, and tests requested.
- •Label the samples with animals' identification
- •Please do not put large tissue specimens in formalin in narrow-necked containers--they go in fresh a lot easier than they come out once fixed!

E-MAIL RESULTS AVAILABLE--We are increasing our reporting by E-mail. To get your E-mail address entered into our system, send an E-mail to: jkammerz@vth.colostate.edu or list your address on your submission forms **very** clearly printed.

WHAT'S INSIDE THIS ISSUE OF LABLINES