The Accreditation Committee of the American Association of Veterinary Laboratory Diagnosticians informed the Veterinary Diagnostic Laboratory in early November it had voted to grant full accreditation status through 2017. AA VLDA accreditation is based on an on-site audit which follows a prolonged detailed review of laboratory documents, including the quality-assurance and policy manuals, system standard operating procedures, training records, financial status and information technology policies. Four members of the accreditation committee and special auditors conducted the audit, reviewing equipment, facilities, safety procedures and compliance with the laboratory’s system, as well as testing SOPs in an intense four-day inspection. They also met with members of the laboratory’s external advisory committee, head of the Microbiology, Immunology and Pathology department, the college dean and the university president.

According to VDL Director Barb Powers, “AA VLDA accreditation assures the clients of the laboratory that results are accurate and of the highest quality by assuring the competence of personnel, the proper function of the facilities and equipment and appropriate documentation of all laboratory tests and processes.”

AA VLDA Accreditation is based on the internationally recognized ISO/IEC 17025 standard and is consistent with the World Organization for Animal Health (OIE) Quality Standard for Veterinary Laboratories. Accreditation is a formal recognition of the competency of laboratories and increases client confidence in diagnostic test results. In order to further demonstrate technical competence between accreditation assessments, personnel from accredited laboratories are also required to participate in relevant proficiency testing programs. Accreditation contributes to continuous improvement and is a management tool that can be used to increase laboratory efficiency, which is critical in times of emergency or limited funding. Laboratories participating in the USDA’s National Animal Health Laboratory Network may be involved in surveillance for early detection of foreign animal disease, surge testing during an outbreak, and testing samples during the outbreak recovery phase. As such, there must be a high degree of confidence in the quality of the laboratories and associated test results. Accreditation is also necessary for assurance and acceptance of test results for live animal export to other countries.

### Lab Updates

Veterinary Diagnostic Lab Achieves Full, All-Species AAVLDA Accreditation

The Accreditation Committee of the American Association of Veterinary Laboratory Diagnosticians informed the Veterinary Diagnostic Laboratory in November it had voted to grant full accreditation status through 2017. AAVLDA accreditation is based on an on-site audit which follows a prolonged detailed review of laboratory documents, including the quality-assurance and policy manuals, system standard operating procedures, training records, financial status and information technology policies. Four members of the accreditation committee and special auditors conducted the audit, reviewing equipment, facilities, safety procedures and compliance with the laboratory’s system, as well as testing SOPs in an intense four-day inspection. They also met with members of the laboratory’s external advisory committee, head of the Microbiology, Immunology and Pathology department, the college dean and the university president.

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### Diagnostic Snapshot

Between June 1 and Sept. 25, CSU VDL veterinarians diagnosed:

- Anthrax (cattle): 31 tested, 7 positive
- Rabies (bats, skunks, bison and raccoons): 253 tested, 29 positive
- Epizootic Hemorrhagic Disease (cattle and yak): 59 tested, 16 positive
- Avian flu (birds): 694 tested, 0 positive
- Plague (wildlife and pets): 10 tested, 2 positive
- Tularemia (wildlife): 7 tested, 1 positive
- Piroplasmosis (horses): 150 tested, 0 positive

### Anthrax Outbreak in Northeast Colorado is Contained

CSU’s Veterinary Diagnostic Lab has tested 31 head of cattle for anthrax in relation to the July and August outbreak in Logan County. Seven animals have been confirmed positive. This first identified anthrax case in Colorado in 31 years, according to the Colorado Department of Agriculture, resulted in no infected cattle leaving the premises or entering the food chain, said State Veterinarian Keith Roehr. “In all, approximately 55 cattle, mainly adult cows, died due to the outbreak; four Logan County premises were quarantined and subsequently released after fulfilling disease control requirements. A cooperative effort among the owners, private veterinarian, CSU VDL, emergency management entities, regional and state public health agencies, and local/state/federal officials was paramount to the effective response of this outbreak.” Sunny Geiser-Novotny, USDA Area Veterinarian in Charge, commended CSU VDL for its role in assisting the containment through timely and accurate diagnosis. “The laboratory did an excellent job in diagnosing the anthrax case, providing a confirmed diagnosis the same day as receiving a recently dead bovine carcass and providing same-day results on all samples submitted afterwards for over three weeks.”
Four CSU VDL case studies, involving three goats from Mesa, Montrose and Prowers counties in Colorado and one calf from Emery County, Utah, demonstrate the need to consider copper deficiency in diagnoses.

In one of the cases, a local veterinarian submitted tissues from a field necropsy. The others were submitted to this laboratory as necropsy cases for complete workup. Complete clinical histories were not available for all cases; however, common features were observed in all four cases: CNS signs, an extreme level of care after they were severely affected and a history of CNS difficulties, recumbency and death in other animals, as well.

**CLINICAL HISTORY AND HEPATIC COPPER LEVELS**

**Case 1.** A 32-pound 4-month-old Boer goat was presented with a history of abnormal gait leading to recumbency. Hepatic copper levels were extremely low at 15.4 ppm (DW).

**Case 2.** Tissues, including brain tissue, were submitted from a 3-month-old male kid with a clinical history of “fading away.” No spinal cord was submitted for microscopic examination. Hepatic copper levels from this animal were 79.9 ppm (DW), which is considered to be on the low end of normal. Deficiencies are generally observed in animals with less than 40 ppm copper.

**Case 3.** A 1.5-year-old Boer goat was presented with a history of recumbency for about 8 months. Hepatic copper levels were 30.4 ppm (DW), and molybdenum levels were slightly increased.

**Case 4.** A 3-month-old crossbred bovine calf was presented in a recumbent condition that had lasted for six weeks. The history indicated similar cases reported from this ranch previously. The hepatic copper levels on this animal were 29 ppm (DW). A water-supply trace mineral analysis indicated increased levels of potassium, magnesium and sulfur. Molecular procedures failed to demonstrate BVD viral genetic material in the tissues submitted.

The significant CNS lesions found in all of these animals included thinning of the granular layer and degeneration or necrosis of Purkinje cells in the cerebellum. Spinal cord lesions included severe Wallerian degeneration within the white matter and scattered multifocal neuronal degeneration within the gray matter.

**DIAGNOSTIC INTERPRETATION AND DISCUSSION**

Two types of copper deficiency generally occur:

- **Primary copper deficiency** occurs when dietary feeds contain less than about 400 ppm copper.
- **Secondary copper deficiency** occurs when excessive amounts of molybdenum, sulfate, zinc or iron in the feed or water interfere with copper absorption. Cattle will usually perform normally when the copper to molybdenum ratio is from 5-to-1 to 10-to-1; however, when the copper-to-molybdenum level falls below 2-to-1, the animal is in danger of clinically evident copper deficiency.

With a few exceptions, plants are the primary source of dietary copper of grazing animals. Levels of copper in plants are important to ruminants because the utilization of copper is tied closely to that of molybdenum. Copper levels in plants generally reflect the copper status of the soils on which they are grown, and animals grazed in parts of the intermountain west suffer...
from molybdenosis, molybdenum-induced copper deficiency. This antagonism is unique to ruminants and is provided by the formation of copper complexes with thiomolybdate, which are poorly absorbed from the intestines. This whole series of events is responsible for reducing copper availability at the local tissue level.

Copper is required for catalytic activity of enzymes that are essential for neuronal function and other cellular activities. Species and breed differences, pregnancy, plant/soil relationships, fiber content of the diet and seasonal conditions will govern nutritional requirements. Aspects of copper metabolism differ significantly between sheep, goats and, likely, other species.

The effects of copper deficiency on the CNS occur in two ways: in utero and during early neonatal life. The congenital disease is commonly called “swayback,” and the delayed form is called “enzootic ataxia,” at least as described in lambs, kid goats and piglets. As veterinary diagnosticians and pathologists, it is important to consider a differential diagnosis as we proceed toward a definitive diagnosis in any case workup. Determining a definitive diagnosis usually requires multiple diagnostic procedures and tests. In these particular cases, we used histopathologic evaluation, toxicology determination of trace minerals and molecular procedures for determining the presence or absence of infectious agents. The initial differential diagnosis for the goats included caprine-arthritis-encephalitis (CAE) complex, various forms of trauma or presence of abscesses, meningitis-encephalitis, nutritional myopathies, toxic neuropathy (due to organophosphates, lead or other heavy metals) and trace mineral deficiencies. For the calf in case No. 4, we also considered bovine virus diarrhea (BVD).

Initially, histopathologic evaluation of the brain or spinal cord revealed lesions suggestive of copper deficiency. Various tests were ordered for each of the cases to help confirm this particular diagnosis. In all cases copper levels were considered to be low and the histologic lesions consistent with that of copper deficiency. Other histopathologic lesions or positive laboratory findings were sufficiently lacking to incriminate other diseases on our differential list.

Neonatal copper deficiency has been most commonly described in lambs, kid goats and piglets. However, copper deficiency has been diagnosed in other animals as well, with clinical signs that may include ill thrift, poor growth, rough hair coat, faded hair color, diarrhea, lameness, depraved appetites and infertility. In cases with similar histories to those described in these four cases, copper deficiency should certainly be on your differential diagnosis list.

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Animal Species and Sex</th>
<th>Animal’s Age</th>
<th>Clinical Presentation</th>
<th>CNS Lesion Distribution</th>
<th>Hepatic Cu levels*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female goat</td>
<td>4 months</td>
<td>Difficulty walking</td>
<td>Cerebellum and spinal cord</td>
<td>15.4 ppm (DW)</td>
</tr>
<tr>
<td>2</td>
<td>Male goat</td>
<td>3 months</td>
<td>“Fade-away” CNS signs</td>
<td>Cerebellum; no spinal cord present</td>
<td>79.9 ppm (DW)</td>
</tr>
<tr>
<td>3</td>
<td>Female goat</td>
<td>1.5 years</td>
<td>Recumbent for 8 months</td>
<td>Cerebellum and spinal cord</td>
<td>30.4 ppm (DW)</td>
</tr>
<tr>
<td>4</td>
<td>Female calf</td>
<td>3 months</td>
<td>Recumbent for 6 weeks</td>
<td>Cerebellum and spinal cord</td>
<td>29.0 ppm (DW)</td>
</tr>
</tbody>
</table>

* Less than 35-40 ppm (µg/g) is considered deficient.
CSU VDL in the Field: Disease Updates

First Case of Epizootic Hemorrhagic Disease Described In Colorado Yaks

In August, CSU VDL’s Virology Section diagnosed the first case of epizootic hemorrhagic disease (EHD) in Colorado yaks. Eight yak have been diagnosed positive from five premises in Larimer and Alamosa counties. The drought may have some effect on the number of cases; as of early October, Nebraska reportedly had confirmed the death of 4,768 deer. Seemingly more cattle than usual were diagnosed in a number of midwestern states, with Nebraska having more than the usual number of cases. It’s been a bad year across the country for this disease, especially in deer. It’s actually likely here every year, and likely it’s in many different animals.

SPREAD BY BITING MIDGES

EHD is an acute, infectious, non-contagious viral disease affecting wild and domestic ruminants. The causative epizootic hemorrhagic disease virus (EHDV) belongs to the family Reoviridae, genus Orbivirus, and shares many morphological and structural characteristics with the other members of the genus, such as bluetongue virus, African horse sickness virus and equine encephalitis viruses. It is most commonly found in deer, especially whitetail deer in the non-western U.S. regions and southern Canada. Mule deer are also known to be susceptible, along with antelope and elk.

Like bluetongue disease, the mode of transmission is via a Culicoides biting midge. Most outbreaks occur between August and October and cease with the onset of frost since no animal-to-animal transmission occurs.

Clinical symptoms of note in the known infected yak included fever, depression, inappetance, ocular and nasal discharge, dyspnea suggestive of respiratory infection, ulcerative gingivitis of the dental pad with mild bleeding from the mouth, along with some variable lameness. Infected cattle show similar clinical symptoms. Infection in cattle leads to milk-production decreases and, if it occurs at a critical time of about 70 to 120 days of gestation in cattle, abortions or birth defects.

Diagnosis can best be confirmed by polymerase chain reaction (PCR) testing on whole blood from the live animal. If a blood sample is not available, spleen would be the PCR tissue submission of choice. Liver, heart, lung and kidney tissues obtained at necropsy may be helpful, as well. Serologic AGID testing is also available. PCR was primarily used to confirm the Colorado diagnoses. A portion of the cases demonstrated positive AGID testing for both EHD and bluetongue. Genotyping of the EHD virus determined the positive cases here were type 2.

No specific successful treatment nor vaccine for EHD are available. Supportive treatment with supplemental feed and methods of combating dehydration are helpful in affected animals that go off feed and water or those showing significant lameness. Antibiotics to prevent secondary bacterial infection and anti-inflammatory drugs would be in order, preferably NSAIDs for the obvious reasons of pregnancy considerations as well as immune-response effects.

— Charlie Davis, DVM, CSU VDL Lab Coordinator, and Hana Van Campen, DVM, PhD, DACVM, CSU VDL Virology Section Head
Feline Virology

FIP DFA: A New Addition to FIP Diagnostic Tools

D

iagnosis of feline infectious peritonitis (FIP) is notoriously difficult due to the highly complex nature of the disease and its various manifestations. CSU VDL offers several options to aid veterinarians:

- Protein electrophoresis, which detects the polyclonal gammapathy characteristic of some FIP cases.
- An FIP RT-PCR, which detects FIP viral RNA.
- An FIP serology test (antibody titer) determined by IFA (FIP IFA).
- An immunohistochemistry stain on formalin fixed tissue, which detects viral antigen in pathologic lesions.

NEW OPTION FOR DIAGNOSIS

To aid in FIP diagnosis, CSU VDL now offers an FIP direct fluorescent antibody (DFA) test. This test can be performed on any fresh or fresh/frozen tissue, including biopsy tissues and lymph node aspirates, as well as on cells found in abdominal and pleural effusions.

Detecting FIP antigen in cells and tissues by DFA staining can accurately predict FIP infection. A positive FIP DFA staining on a sample will provide a definitive FIP diagnosis; however, a negative FIP DFA result does not exclude the possibility of FIP. In cases of the “wet” form of FIP, cells in abdominal or thoracic effusions are not excluded for FIP. In cases of the “wet” form of FIP, the tissues most likely to contain FIP antigen are granulomas on liver and spleen.

SUBMISSIONS

FIP DFA tests costs $20 per sample. Submit effusions by one of two options:

- Make a smear of the effusion fluid at the clinic, which will then be stained by FIP DFA at the lab.
- Submit the effusion in a purple-topped tube (EDTA). In this option, cells in the effusion will be concentrated and placed on a slide using a cytospin centrifuge here in the CSU Diagnostic Medicine Center’s Clinical Pathology section for an additional charge of $6.75 per sample.

STILL A PLACE FOR NECROPSY?

Researchers from University of California Davis’ veterinary hospital updated their two-decade series of studies to help determine how accurately ante-mortem diagnoses match the post-mortem necropsy results—in the increasingly rare cases those procedures are now done.

Their follow-up retrospective on the records of 148 hospitalized dogs that died during 2009 showed the proportion of discrepancies was significantly lower (at P<0.001) in 2009, at 14.9 percent, when compared to both 1999 and 1989, which were 37 percent and 39.8 percent, respectively. By 2009, necropsies had grown significantly less common, with only 21.4 percent of dead or euthanized dogs necropsied, compared to 48.4 percent and 58.9 percent for ’99 and ’99, respectively.

The authors cautioned that their results likely overstate the number of necropsies done in general practice, because they come not only from a teaching hospital, where necropsies are known to be more common, but from an institution that fully subsidized the cost, which is bound to further increase their likelihood.

Still, they caution, necropsy plays an important role. VDL Pathologist Colleen Duncan agrees. In food-animal medicine, it remains an important tool to evaluate production, influence production-management decisions and monitor herd health status. In small animal medicine, post-mortem examination is an important tool for teaching and follow-up. At the same time, Duncan says, it’s important to remember one possible explanation for the declining rate of discrepancy in the Davis study is that pathology has improved in ante-mortem applications as well, expanding the diagnostician’s options to diagnose, and possibly to treat successfully.

For further information please call Christina Gates or Hana Van Campen at (970) 297-1287.

SOURCES

Food Animal Production Medicine

Tackling a $1 Billion-Plus Problem: CSU VDL Refocuses on Milk Testing

Mastitis is one of the largest disease costs on dairy farms. Subclinical mastitis—cases in which dairy cattle contract a chronic udder infection not severe enough to show symptoms but capable of decreasing milk production and quality over the longterm—was estimated in 1999 to cost the U.S. dairy industry $1 billion dollars a year. More loss is attributed to the more-apparent clinical mastitis.

CSU VDL now offers many milk testing options to help control this costly problem through improved mastitis identification, including bacteria identification and Mycoplasma culturing. This will include all bacteria being identified to at least the genus level and is available for composite milk samples. CSU has a “contagious milk culturing” option in which we look for only Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma spp. We also have options for just bacteria identification or solely Mycoplasma culturing.

Identifying Mycoplasma can be done not only through culturing but also by PCR. The PCR option is great for taking composite milk samples such as tanks or string samples and using PCR to see if Mycoplasma is detected. At that point dairies can submit individual samples to determine which cattle are infected by either PCR or culture. When Mycoplasma is found by using the culture option it can be speciated by a PCR confirmation test that will give the species and ensure that the Mycoplasma found is not Acholeplasma laidlawii which is generally considered not to be a cause of mastitis.

MILK-QUALITY TESTING OPTIONS

CSU has many other milk quality testing options available. The “Milk Coliform Count” can help conclude if there is an issue in the udders of cows or if there are unsanitary milking practices. The “Milk Standard Plate Count” is the official reference method for determining bacterial numbers in raw milk from the Pasteurized Milk Ordinance. The “Milk Laboratory Pasteurized Milk Ordinance” helps identify organisms that survive pasteurization. Typical mastitis-causing organisms are not able to survive pasteurization. High LPC counts are usually associated with unclean equipment or improper sanitizing practices. The “Milk Preliminary Incubation” (PI) looks for psychrotrophic bacteria, meaning bacteria that grow well in cold conditions, and not mastitis-producing bacteria. The PI is a test to help determine the shelf-life and monitor sanitization practices on farms. CSU offers all these tests individually or all together as a “Milk Quality Test.”

If samples are going to be delivered to the lab within two days of collection, they can be refrigerated and transported in a cooler. If the samples will not reach the lab within two days, they should be frozen and shipped overnight. CSU currently offers a courier service in the Fort Collins area. If you are not located in the Fort Collins area and are interested in a courier service, please contact us.

Sources


Nitrate Testing Through CSU VDL Rocky Ford

- If testing bales, try to core 10 percent of bales
- If field sampling, mix one sample from all quadrants plus center, using a “W” or “X” pattern.
- Cost $10 per sample.
  No additional accession fee or out-of-state fee.
- Many samples reported out same day during work week; virtually all within 24 hours of receipt.

Nitrate Testing Reminders from CSU VDL Rocky Ford Branch Laboratory

Accessions for forage nitrate testing have been running as many as 15 to 20 sample requests per day, says Rocky Ford Director Gene Niles. “Luckily, the vast majority are within normal limits,” Niles says, “But we are finding some that are way too high.”

As livestock producers increase nitrate testing in response to quality issues affected by the summer drought, Niles suggests they keep these points in mind:

- The test result numbers producers receive are only going to be as good as the samples the lab receives. “We need a sample that is representative of the field, not just a single grab sample. We’ll take anything, because it gives us a start, but we certainly prefer core samples of several bales or samples from throughout the field.
- Understand units for interpretation. Nitrites are reported out as either nitrate, nitrate nitrogen or potassium nitrate, and each of those differ. If you have questions about interpretation, call the lab.
- Don’t graze corn, sunflowers, sorghum or millet that has been abandoned due to drought or hail stress without testing for nitrates first.
- Test any of this year’s forages to be fed.
- Also test livestock water sources for nitrates. Toxicity results from the combined daily total from both feed and water. Feeds considered safe under normal conditions could cause toxicity poisoning when combined with water moderate or high in nitrate.
- Bear in mind accurately sampling standing forage for nitrate toxicity is difficult, since it can vary widely within a field.
as other arrangements may be possible depending on volume, amount, and location. If you are planning to use FedEx for a delivery service you also may want to contact us for information on how we can reduce your shipping costs. Contact the laboratory at 970-297-1281 or dlab@colostate.edu.

CSU also now offers a bovine pregnancy test. Accurate and early detection of pregnancy is invaluable for both dairies and beef producers. The test can determine pregnancy as early as 28 days after breeding, and a cow is identified as negative as soon as 60 days post calving without interference from the previous pregnancy. The test is exceptionally accurate with an "open" result that is 99 percent reliable. Besides using serum, the pregnancy test is now available using milk, making sample collection easier and giving more options for testing. For example you can collect a single milk sample and run the pregnancy test, Johnes, bacteria ID, mycoplasma, and Milk Quality Tests from one large vial of the same milk sample. The test can also be run using a Dairy Herd Improvement Association (DHIA) milk sample, because the preservative tablet does not interfere with the test. This means you can collect for your DHIA and pregnancy tests at the same time. Call us for details.

When selecting a milk-testing lab, keep in mind that not all labs are the same. Shedding contagious mastitis bacteria is not predictable; many infected cows can shed very small numbers of bacteria. The VDL takes many steps to ensure the tests we run are the most sensitive and accurate, and we do not cut corners that would jeopardize the quality of the results. CSU VDL also has some of the most advanced diagnostic testing equipment and highly educated technicians. The lab is also fully accredited by the American Association of Veterinary Laboratory Diagnosticians, meeting standards based on ISO17025 / OIE, and is a member of the National Animal Health Laboratory Network. When choosing CSU for your Veterinary Diagnostic testing you can be sure you are getting the best results from the sample that has been submitted.

For recommendations on screening for mastitis and how to collect aseptically, see "Milk Culture Screen Programs for Detection of Contagious Mastitis," available on the Web at: www.cvmbs.colostate.edu/ilm/proinfo/cdn/2001articles/CDNsept01insert.pdf
CSU VDL in the Field: Case Study

Sulfur-Associated Bovine Polioencephalomalacia

Approximately 1000 head of bred Angus calves were pastured on sub-irrigated land in a mountain river valley along the Colorado-Utah border of eastern Utah. This was a large pasture containing two water sources, one, a spring fed pond and a second pond created by digging into the sub-irrigated ground and allowing the hole to fill with groundwater. During a period of approximately five days, six animals either died or became recumbent. An 800 pound heifer calf was presented to the CSU Western Slope Veterinary Diagnostic Laboratory to determine the cause of death of this animal and to investigate the disease outbreak.

NECROPSY AND LABORATORY FINDINGS:

Necropsy findings in this bred-heifer calf provided little information regarding the cause of death. Numerous tissues, including brain, were collected and submitted for histopathologic evaluation, and bacterial culture. Samples of rumen contents were also submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory to determine if there were toxic plants or seeds present in the rumen material. At this point, polioencephalomalacia was considered as the most likely cause of death in this animal. A differential diagnosis would include meningoencephalitis caused by Histophilus somnus or Listeria monocytogenes, rabies, heavy metal toxicity or possibly various toxic plants.

Histopathologic examination of the brain revealed laminar cortical necrosis/malacia, necrosis and loss of neurons and edema within the cerebral neurophil. These lesions are typical of bovine polioencephalomalacia and are illustrated in figures 1 and 2. At this point, as requested by this laboratory, another calves’ head was submitted and the brain processed for histopathologic examination. The second brain contained typical lesions of bovine polioencephalomalacia as well.

Water samples were also requested from both of the ponds/watering holes. The water samples were submitted to the CSU Rocky Ford facility for a Livestock Water Screen to determine, among other things, the level of water sulfate. The sulfate level in the spring fed pond was 670 ppm and the sulfate level in the second pond/waterhole was 5000 ppm. The sodium level in the pond/waterhole was also elevated to 1560 ppm, as compared to 131 ppm in the spring fed pond.

DIAGNOSTIC INTERPRETATION:

The causes of polioencephalomalacia may include thiamine deficiency, exposure to high levels of sulfur and/or salt (sodium ion), exposure to lead or other heavy metals and perhaps other unknown causes. In recent years there has been an increasing frequency of sulfur-associated polioencephalomalacia reported in the literature. High dietary levels of sulfur have been incriminated for causing this disease1. Cattle in central Saskatchewan were exposed to high levels of sulfur in the drinking water (3400 ppm sulfate) which caused lesions of classical polioencephalomalacia 2 as well. Ruminal sulfur compounds may be directly related to cellular deficiencies of thiamine. Sulfide, an intermediate in the reduction of sulfate, can cleave thiamine into pyrimidine and thiazole constituents thereby rendering it inactive3, 4. Hydrogen sulfide and free sulfide radicals inhibit electron transport.

SOURCES


(left) Necrosis of neurons and laminar cortical necrosis. (right) Necrosis of neurons and perivascular edema.
during the cellular oxidative process and can cause cellular anoxia and death. This can lead to acute respiratory failure as well. Direct salt poisoning may also cause polioencephalomalacia; however, lesions are generally seen later in the disease and malacia is not always observed.

The scenario described in this particular case is not uncommonly seen in diagnostic laboratories throughout the Western United States. The presence of alkaline soils in Western Colorado and Eastern Utah are commonly observed making this scenario more plausible, especially during years of drought.

**New Virology**

**New Rapid BVD PI Test Available**

According to studies conducted in North America and Europe, bovine viral diarrhea virus (BVDV) is one of the most economically damaging diseases in both feedlot and dairy cattle. On average, BVDV costs beef producers approximately $42 per animal and approximately $48 per animal in dairies. These losses amount to extremely large sums when considering the number of beef and dairy cattle in the United States, and significantly affect both small and large-scale producers.

Persistently-infected (PI) cattle are by far the major source of BVDV. While transiently-infected animals can spread the disease to other animals, they shed much lower levels of virus and for a much shorter period than PI cattle. A PI animal develops when the dam becomes infected with BVDV within the 2nd to 4th month of pregnancy. If this fetus survives until birth, it will be born tolerant to its infecting BVDV strain and will shed large numbers of that virus for its lifetime. This animal is then a constant source of infection to other animals.

One of the most important ways to avoid losses due to BVDV is to ensure that PI animals are removed or never included in a cattle population. Several diagnostic tests using serum or ear notch samples can determine if an animal is a PI animal. Depending upon when a sample is received, most of these tests have a turnaround time of 24 hours to 1 week. The CSU Veterinary Diagnostic Laboratory is now offering a new test that has been developed by IDEXX Laboratories using their SNAP technology, similar to that used in their FeLV/FIV and Parvovirus SNAP tests. It will significantly decrease the turnaround time for BVD-PI status test results. The tests previously available for testing BVD PI-status were the IDEXX BVD antigen-capture ELISA, BVD immuno-histochemistry (IHC), BVD RT PCR and virus isolation. Whereas our BVD antigen-capture ELISA test is run once weekly on Wednesday afternoons, a serum or ear notch sample that is received by noon Monday through Thursday that is tested using the SNAP BVD test can be run and reported with a 24-hour period. The BVD SNAP test is considered to be highly comparable to the BVD antigen-capture ELISA test in accuracy and detects both type-1 and type-2 BVDV 99.1 to 100% of the time. The BVD SNAP test cost is $10.00 per sample. The BVD antigen-capture ELISA, IHC, PCR and virus isolation will also remain available for those who prefer these test methods.

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**SOURCES**


A Roundup of VDL Faculty Research


VDL Endocrinology and Special Serology Lab Supervisor Michael Lappin co-authored a Journal of Feline Medicine and Surgery editorial introduction to a comprehensive new review on the numerous clinical and diagnostic challenges posed by Bartonella infection in cats. Since the rediscovery of the genus in the early 1990s, researchers have continued to realize those challenges are much more complex than currently appreciated, in both veterinary and human medicine. The expanding number of documented reservoir hosts, an increasing number of arthropod vectors, the diversity of Bartonella species and subspecies, geographic patterns, variation in pathogenicity, infection with a “non-reservoir adapted” species, and occupational risk of infection due to frequent exposure all contribute to a continuing discovery and rediscovery of this oft-debated disease. “We’ve a way to go in our understanding of bartonellosis...,” Lappin writes.


A multi-institutional USDA study involving VDL Pathologist Terry Spraker tested the diagnostic accuracy of rectal mucosa biopsy in cases of chronic wasting disease (CWD) among captive white-tailed deer. It demonstrated selective use of antemortem rectal biopsy sample testing could provide valuable information during disease investigations of CWD-suspect deer herds. The study compared the immunohistochemical detection of disease-associated prion protein in postmortem rectal mucosa biopsy samples to the CWD status of each deer, as determined by immunodiagnostic evaluations of the brainstem at the obex, the medial retropharyngeal lymph node, and the palatine tonsil. It found diagnostic sensitivity of the test ranged from 63 percent to 100 percent; the pooled estimate of sensitivity was 68 percent with a 95 percent confidence limit of 49 percent and 82 percent. However, diagnostic sensitivity depended on the genotype at prion protein gene (PRNP) codon 96 and on the disease’s progression according to obex grade. Sensitivity was 76 percent, with 95 percent confidence levels of 49 percent and 91 percent for 96GG deer, but it fell to only 42 percent (95 percent confidence levels of 13 percent and 79 percent) for 96GS deer. Sensitivity was only 36 percent for deer in the earliest stage of disease (obex grade 0) but was 100 percent for deer in the last 2 stages of preclinical disease (obex grades 3 and 4). The overall diagnostic specificity was 99.8 percent.


VDL Director Barb Powers assisted CSU Animal Cancer Center colleagues Jenna Burton and Barbara Biller to conduct this first published assessment of outcome and prognostic factors for hemangiosarcoma (HSA) of the tongue in dogs. Although the spleen is the most common primary site of HSA, less commonly, HSA also arises from tissues such as the tongue. However, case reports of lingual HSA are sparse in the veterinary literature. The CSU team searched histopathology submissions to CSU’s VDL to eventually narrow a group of 20 dogs for which a histologic diagnosis of HSA of the tongue was made between January 1996 and December 2011. Dr. Powers retrospectively confirmed the diagnosis of HSA and graded the tumors based on previously established criteria for HSA. They found prognostic factors significantly associated with increased survival included small tumor size and absence of clinical signs of an oral mass at the time of diagnosis. Dogs with HSA confined to the tongue may have a better prognosis compared with HSA in other organs, the team concluded.

CSU VDL Avian Diagnostics and BSL3 Operations Section Head Kristy Pabilonia designed and supervised this epidemiologic characterization of Colorado backyard poultry flocks to gather information on general flock characteristics, human movement of birds, human-bird interaction, biosecurity practices, and flock health. The results suggest that backyard poultry flocks in Colorado are small-sized flocks (68.6 percent of flocks had less than 50 birds); consist primarily of layer chickens (85.49 percent of flocks), show chickens (32.18 percent of flocks), and waterfowl (34.07 percent of flocks); and are primarily owned for food (meat or egg) production for the family (86.44 percent) or as pet or hobby birds (42.27 percent). The backyard flock environment may promote bird-to-bird transmission as well as bird-to-human transmission of infectious disease. Birds are primarily housed with free access to the outside (96.85 percent), and many are moved from the home premises (46.06 percent within 1 yr). Human contact with backyard flocks is high, biosecurity practices are minimal, and bird health is negatively impacted by increased movement events. Increased knowledge of backyard bird characteristics and associated management practices can provide guidelines for the development of measures to decrease disease transmission between bird populations, decrease disease transmission from birds to humans, and increase the overall health of backyard birds.


VDL Bacteriology Section Head Doreene Hyatt and the Bacteriology Section provided culture and antimicrobial susceptibility results for a study examining the role of Corynebacterium spp. in the pathogenesis of canine and feline otitis externa/media and their appropriate antimicrobial therapy. The retrospective study targeted cultures positive for Corynebacterium, finding them part of mixed microbial populations in 79 of 81 cultures. Corynebacterium spp. pathogenicity was highly questionable because of their almost invariable presence with other microbes and the observation that Corynebacterium spp. usually disappear from the ear with resolution of other infections, even when the Corynebacterium spp. are resistant to the prescribed antibiotics. However, two of the 81 cultures came from two canine ears where Corynebacterium spp. may have been pathogenic. Antimicrobial sensitivities for Corynebacterium spp. were available for 54 isolates. Most isolates were susceptible to chloramphenicol (53/54), amikacin (50/54), tetracycline (50/54), gentamicin (46/54) and enrofloxacin (32/54). Among those antibiotics available in otic products, gentamicin and enrofloxacin would be rational choices for the empirical, topical therapy of Corynebacterium spp.

CSU VDL Virology Section Head Hana Van Campen and Pathology Resident Brett Webb participated in a study that first generated BVDV Persistently Infected fetuses by intranasal inoculation of pregnant heifers with ncp BVDV, and then collected those PI and uninfected control fetuses via C-section at 82, 89, 97, 192, and 245 days of gestation. They then examined those fetuses to delineate timing of the development of innate immune responses in the fetus and placenta during establishment of persistent infection.

They found significant up-regulation of mRNA encoding cytosolic dsRNA sensors – RIG-I and MDA5 – was detected on days 82 through 192. Detection of viral dsRNA by cytosolic sensors leads to the stimulation of ISGs, which was reflected in significant up-regulation of ISG15 mRNA in fetal blood on days 89, 97 and 192. No difference in IFN-α and IFN-β mRNA concentration was found in fetal blood or caruncular tissue, while a significant increase in both IFN-α and IFN-β mRNA was seen in cotyledons from PI fetuses on day 192. Fetuses respond to early gestational ncp BVDV infection by induction of the type I IFN pathway, resulting in chronic up-regulation of ISGs. The innate immune response might partially curtail viral replication in PI fetuses, but is not able to eliminate the virus in the absence of a virus-specific adaptive immune response.

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Check this quick review of recommended fecal amounts for parasite analysis:

- Fecal floats and Cryptosporidium/Giardia IFAs are best performed using 3 grams of feces, or roughly a walnut-sized amount. A minimum 1 gram of feces may be used without significantly altering test accuracy. We recognize diarrhetic samples are not easily collected, but sending as much as possible is helpful.
- Baermann set ups ideally contain at least 5 grams of feces, or roughly the size of a kiwi; more is always welcome.
- Direct wet mounts and feline T. foetus cultures only require a peppercorn-sized amount of feces. Mixing three parts of fresh feces with two parts of normal saline before shipping at room temperature helps preserve trophozoites during transport. Removing as much cat litter or extraneous debris from the feces before mixing with saline improves the sample quality. We will take the required amount of sample from this mixture upon arrival.

Fecal screens combine three separate tests: a fecal float, a direct wet mount, and a Cryptosporidium/Giardia IFA. Obtaining an ideal 6- to 7-gram sample for the fecal screen is a common challenge. A minimum 2 grams of feces for all three tests is acceptable, as the direct wet mount doesn’t require much sample. If the total amount after the wet mount is less than 2 grams, we prefer to concentrate the rest of the feces toward the fecal float or the Cryptosporidium/Giardia IFA. If the animal is unable to produce the recommended amount, please indicate your test preference in order of importance.

Feline T. foetus PCR samples require 1 gram of feces. This can be sent on ice without problem.

Submitting samples for multiple test combinations such as fecal screens and feline T. foetus culture or PCR can be confusing. Remember that because icing or refrigerating the sample kills trophozoites that would be detected upon direct wet mount and feline T. foetus culture, if ever in doubt, send the feces at room temperature.
Get to Know the Laboratory

New Members Join the Lab Team

Charlie Davis. DVM, born in Monte Vista, was raised on a potato, grain and livestock farm in the San Luis Valley of south central Colorado. Following graduation from CSU College of Veterinary Medicine in 1967, he practiced in a mixed practice in Durango for three years, an equine and cattle practice in Littleton for one year and returned to Monte Vista in 1971, where he worked in a mixed practice for two years before building his own clinic and starting his own mixed practice there in 1973. He spent one year in Lincoln, Neb., doing contract clinical research with large animal biological development before moving to Fort Collins in 1998, where the contract clinical biological research with cattle, swine, sheep and some small animal veterinary pharmaceutical development work continued until a change of corporate ownership.

Charlie is a member of organized veterinary medicine at the local, state and national levels, served eight years on the Colorado State Board of Veterinary Medicine, represented the Colorado Veterinary Medical Association on the accreditation committee for veterinary technology programs in the state for five years, is a past member of the CSU diagnostic lab advisory board and also is a member of the Colorado Cattlemen’s Association as well as the Colorado Wool Growers Association. Along with his wife of 48 years, Barbara, they enjoy following numerous sports at all levels, dining out while avoiding chains if possible, viewing and photographing wildlife in our mountains, traveling mainly in the United States and doing things that provide new and unique experiences. Seems of utmost importance at present is keeping up with the families of a son and daughter who have each provided two grandchildren that seem to occupy a majority of time, obviously well spent.

Having been at the Diagnostic Lab for three months as case coordinator, the learning experience has been great but the kindness, assistance and cooperation of everyone from every part of the lab has been special.

Dave Hicks grew up in Yuma, then moved to the northern Colorado area where he completed a bachelor of science degree in Microbiology from CSU in 2008. Since then, he has been working in the milk industry for about four years with a focus on bacterial identification, ELISA testing, and PCR. Dave now works in the bacteriology section of the VDL.

Pete Grabel moved from Peoria, Ill., to Colorado in the summer of 2010 and joined as an administrative assistant with the Veterinary Diagnostic Lab in October of 2012. Before starting at CSU, he repaired computers for two years in Loveland. He hopes to further his education at CSU pursuing computer science courses.

Adrienne Espinosa is a Colorado native. She previously worked at the university with the School of Social Work. She relocated for several years and is excited to be reinstated with the Veterinary Diagnostic Laboratory. She enjoys spending time with her family and taking in the beautiful outdoors.
The College of Veterinary Medicine and Biomedical Sciences welcomed Mark Stetter to the role of Dean on July 1, following the retirement of Lance Perryman, who had led the College for almost 11 years. Stetter, who arrived on campus May 1, has spent his summer meeting with faculty staff, and students; assisting with fundraising efforts; navigating his way around campus; and getting to know the lay-of-the-land at Colorado State University.

“In the short time that I have been here, I’ve been amazed by the diversity of our programs, the dedication of our people, and the fantastic opportunities we have ahead of us,” said Stetter. “Dr. Perryman has done an incredible job and laid the groundwork for our continued success.”

Says the new Dean, “I’m so impressed with the quality and quantity of diagnostic tests that the lab completes every year, and that’s a message I’m taking out to the greater veterinary and scientific community as I attend professional meetings and meet with those who have an interest in that work. We also have begun concerted efforts to educate the public about the Veterinary Diagnostic Laboratory as a public health resource, and the essential services to human and animal health we provide.”

Stetter has met with department heads, research faculty and associate deans to learn more about the College, and to better understand the College’s expansive programs in teaching, research and outreach; and to identify where some of the College’s greatest needs exist. In the months to come, he looks forward to expanding his outreach to CSU’s other colleges and deans, as well as to key collaborators in government and other academic institutions.

“My personal passion is connecting people with animals and nature,” said Stetter. “This College does so much to help animals, help people and help the planet. I’m really looking forward to see how we can develop new solutions to age-old problems.”

Stetter comes to CSU from the Disney Company where he was the Director of Animal Programs. He graduated with his Doctor of Veterinary Medicine from the University of Illinois at Champaign-Urbana in 1988 and then completed an internship in medicine and surgery at the Animal Medical Center in New York. He served as Associate Veterinarian at the Audubon Zoo and the Aquarium of the Americas in New Orleans, and then completed a residency in zoological medicine at the Bronx Zoo/Wildlife Conservation Society. He is a Diplomate of the American College of Zoological Medicine, and served as president of that organization. He is a recent member of the Wildlife Scientific Advisory Board for the Morris Animal Foundation and is founder and President of the Elephant Population Management Program.
Get to Know the Laboratory

New Rocky Ford Director: ‘On the Front Lines of Vet Diagnostics’

CSU VDL Rocky Ford Branch Laboratory’s new Director Gene Niles looks forward to the “front line” challenges presented by steering a regional veterinary diagnostic lab.

“You could say any diagnostic laboratory is on the front line of veterinary medicine,” says the Oklahoma native and Oklahoma State DVM/MS graduate, “but you know a branch lab like Rocky Ford is closer, so it gives the public the opportunity to present animals they probably wouldn’t otherwise take two or three hours to get to a central lab. Those are exactly the type of animals that need to be evaluated to find the index case of any disease.”

Since taking over the lab’s helm in mid September, the former mixed-lab practitioner, toxicology resident and head of the Illinois Department of Agriculture’s regional lab at Centralia has looked forward to building on the reputation of the lab established by the late Jim Kennedy.

“I want to be sure people realize we’re here and we’re going to do whatever we can to improve our ability to help them, using local diagnostics. We want to build upon the excellent name this lab enjoys, and make sure people know that if they have a dead animal they can’t get to a local veterinarian or the local veterinarian realizes it needs to be looked at, that we can get them to the right place.”

“I’ve always thought there are three primary objectives of any veterinary diagnostic lab. First obviously is diagnosing and mitigating animal disease. But second would be providing regulatory services and regulatory testing to intrastate, interstate and international import and export of livestock. This laboratory is doing a lot of testing involving shipping cattle to Russia right now, for example. The third objective would be constant vigilance for diseases of economic importance or foreign animal diseases.”

CSU VDL ON THE ROAD: UPCOMING CONFERENCES, SYMPOSIA AND APPEARANCES

CSU VDL pathologists Terry Spraker and Colleen Duncan will attend the Alaska marine mammal science symposium meeting in Anchorage on Jan. 30.

CSU VDL Pathologist Colleen Duncan will be at the 44th Annual Conference of the International Association for Aquatic Animal Medicine in Sausalito, Calif., April 21 through 26.

Lab Coordinator Charlie Davis and Virology Section Head Hana Van Campen attended cattle producer meetings in Aguilar and Walsenberg. Dr. Van Campen also attended ACVM in San Diego.

CSU VDL pathologists Colleen Duncan, Tawfik Aboella and EJ Ehrhart attended the American College of Veterinary Pathologists annual meeting, Dec. 1 through 5 in Seattle.

VDL Director Barb Powers will attend the Mid-winter meeting of the Colorado Cattlemen’s Association, Jan. 22 and 23 in Denver. She will also be in attendance at the Government Relations meeting of the American Association of Veterinary Laboratory Diagnosticians in Washington, D.C., in March.

Avian Diagnostics and BSL3 Operations Section Head Kristy Pabilonia and Lab Coordinator Charlie Davis will assist at Denver’s National Western Stock Show, Jan. 12 through 27, 2013. The two will observe, respectively, poultry and livestock for contagious disease, assist class superintendents, support show staff veterinarians and assist State Veterinarian Office personnel with regulatory issues. Don’t miss your chance to meet these two faculty members and learn more about the services CSU VDL provides.

Kristy Pabilonia also traveled to Indonesia for a FAS-sponsored lab training course in November, and travels to accreditation committee training, Feb 12 to 14 in Las Vegas, a Live Bird Market Working Group, Feb 20 and 21 in Seattle, and the National Poultry Improvement Plan State Contact Representative Meeting June 18 through 20.

Pathologist Tawfik Aboellail and Dr. Pabilonia took four veterinary students to Egypt on Jan. 9 to give them some International perspective of the profession abroad.

VDL Director Barb Powers; Bacteriology Section Head Doreene Hyatt; microbiologists Christina Weller and Denise Bolte; Chemistry and Toxicology Section Head Dwayne Hamar; Virology Section Head Hana Van Campen; and pathologists Terry Spraker and Chad Frank attended the 55th Annual American Association of Veterinary Laboratory Diagnosticians Annual Meeting in October.
Welcome to our Fall/Winter issue of LabLines. We have a lot of good news to report in this issue that occurred last summer and early fall. Of incredible importance to us is that we achieved accreditation from the American Association of Veterinary Laboratory Diagnosticians for another five years. What this means is detailed on the front page. Also, other big news includes the arrival of the Dean for the College of Veterinary Medicine and Biomedical Sciences, Mark Stetter, and the arrival of the new Director for the Rocky Ford Branch Laboratory, Gene Niles. See inside for information about them.

We had a very busy summer and some of the details are also in this issue regarding the Anthrax outbreak, EHD and interesting cases from the Western Slope.

Also find inside information about new tests for feline infectious peritonitis, BVD and our refocusing on milk testing, which includes a bovine pregnancy test that many may find of great interest.

We are also very happy to welcome new staff to our laboratory in the office and Charlie Davis as our new case coordinator, whom many of you are familiar with and will be seeing out and about in Colorado. We would also like to welcome new residents in the Pathology area, Travis Meuten and April White, as well as in Microbiology, Ashley Malmlov.

Also, many of you may have noticed we have successfully launched our new website and have new procedures for reporting pathology results. Both of these are designed to increase efficiency and provide higher quality service for our clients.

Respectfully,

Barbara E. Powers
BARBARA POWERS, DVM, PHD, DACVP DIRECTOR

Our new website has launched, along with new procedures for reporting pathology results. Both are designed to increase efficiency and provide higher quality service.

Update from the Director