Welcome to the Spring 2014 issue of Diagnostic Veterinary Matters. Since publication of the last issue, we have continued to grapple with the challenges of reduced state and federal funding while maintaining, improving, and even expanding the services we provide veterinarians and animal owners throughout the state. Recently expanded services include testing for calves persistently infected with BVD virus and bovine brucellosis surveillance testing using bulk tank milk (see lab updates section for more information). Improvement of services has involved our continuous tweaking of test methods to generate more timely and accurate test results.

The approved equipment bond represents about 38% of the funds needed for the laboratories to catch up on replacing numerous pieces of equipment whose replacement has been deferred for the past five years. Therefore, your continuous support will be appreciated in the next two years to obtain the remaining bond funds. Additionally, your support will be needed in assuring that the labs’ state funding can keep pace with the higher than average inflation in the cost of biomedical supplies, while maintaining a stable and well-trained workforce.

We recently were able to replace the roof of the Tifton lab (photo on right) using Major Repair and Renovation (MRR) funds provided through the University of Georgia. Future projects including the repair of the Tifton parking lot and addressing the collecting pond erosion problem at the Tifton laboratory utilizing similar MRR funding are planned.

The Athens laboratory continues with implementation of the UGA-developed laboratory information management software package, VetView. The Tifton lab will also be migrating to VetView in FY15.

Backed by our cadre of highly committed faculty and staff, we remain poised to continue providing you with the most complete, highest quality, and timely diagnostic services. We want to thank all of our clients for your business and your continued loyalty to the GVDLS and look forward to our continuous partnership in maintaining and improving animal health and human well-being in Georgia and beyond.

Murray E. Hines II
Jeremiah [Jerry] T. Saliki
Hypocalcemia or low blood calcium concentrations in dairy cattle negatively impact performance and health. Cows that have clinical milk fever are more likely to have other diseases including retained placentas, mastitis, ketosis, and left displaced abomasum (LDA). While the incidence of milk fever has declined to approximately 5% with the use of improved close-up dry cow nutrition, it is estimated that 50% of all dairy cows in the U.S. experience subclinical hypocalcemia. Cows that have subclinical hypocalcemia have lower milk yield, decreased reproductive performance, and increased odds of ketosis and LDA.

Traditionally the calcium status of the dairy cow has been evaluated by collecting a blood sample for analysis of total calcium concentrations. Total calcium represents calcium that is: 1) bound to plasma protein, 2) in an ionic form associated with low molecular weight compounds, and 3) in free ion forms. Only the free ion form of calcium is available to support metabolic functions including muscle contractions, nerve impulse transmission, and blood clotting in addition to its role in bone formation. Ionized calcium measures the free calcium ions which are readily available for these metabolic functions, providing a better estimate of true calcium status. The proportion of total calcium that is ionized is not constant and changes with stage of lactation. Occasionally total serum calcium concentration indicates that the cow with clinical signs of milk fever has a normal serum calcium concentration, but ionized calcium concentration confirms that she is truly in a clinical state of hypocalcemia.

In recent years an economical method for rapidly measuring ionized calcium using electrodes has been developed that makes this tool available to producers as well as researchers. A unit has recently been installed at the University of Georgia Veterinary Diagnostic & Investigational Laboratory in Tifton that measures ionized calcium and magnesium. Concentrations of ionized calcium less than 1 mmol/L are considered to be an indicator of hypocalcemia. In practice, ionized calcium and magnesium can be used to evaluate close-up dry and transition cow nutrition programs as well as diagnose milk fever, subclinical hypocalcemia, or hypomagnesemic tetany that may occur around parturition or during the early lactation period.

**REMEMBERS**

- **Biopsy charges**: At both the TVDIL and the AVDL multiple punch skin biopsies of a generalized skin condition (dermatitis, alopecia) are charged as one submission.
- **Ionized Calcium and Magnesium**: Concentrations of ionized calcium and magnesium are sensitive to changes in blood pH that occur once the sample has been exposed to air or during storage, so it is important to collect and transmit samples to the lab within hours or process samples before shipping to obtain valid results. Samples should be collected in a green top (Lithium Heparin) tube with separator gel, Vacutainer tube, or a “tiger-top” gel-separator tube and allowed to clot. Once the sample has clotted, it should be centrifuged to separate the plasma or serum from the blood clot and refrigerated immediately.

**DO NOT REMOVE THE CAP** from the collection tube because this introduces air into the sample, increasing the pH. The sample should be packaged with an ice pack and shipped for overnight delivery to the laboratory for analysis. The pH of the sample is measured and used to calculate the final result. If you have questions about sample submission, please contact Anita Merrill at 229-386-3340.

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**PERSONNEL HIGHLIGHTS**

### Athens Veterinary Diagnostic Laboratory

- The Athens Veterinary Diagnostic Laboratory recently participated in a USDA negative cohort study for diagnostic tests for two important exotic diseases: lumpy skin disease and contagious bovine pleuropneumonia. In the process, two staff members (Michelle Norris and Sarah Bates) successfully completed a proficiency test for both diseases. Such activities enhance our preparedness to protect animal health by maintaining the ability to diagnose these exotic diseases if they ever occur in the US.
- Jennifer McClain successfully completed the 2013 proficiency tests for foot-and-mouth disease and classical swine fever NAHN ELISA proficiency tests.
- Dr. Doris Miller passed the Food and Mouth Disease and Classical Swine Fever NAHN RT-PCR proficiency tests.
- Dr. Angela Ellis achieved board certification with the American College of Veterinary Pathologists (ACVP) in September 2013.

### Tifton Veterinary Diagnostic and Investigational Laboratory

- Michele Coarsey, Candice Jackson and Jill Johnson passed the Foot and Mouth Disease and Classical Swine Fever NAHN RT-PCR proficiency tests.
- TVDIL Serology staff passed the Johne’s ELISA and Anaplasma cELISA proficiency tests.
- Dr. Sree Rajeev won the 2013 AAVDL-administered ring trial test for equine herpesvirus.
- Dr. Angela Ellis achieved board certification with the American College of Veterinary Pathologists (ACVP) in September 2013.

**REMARKS**

- **The Clinical Pathology section at the TVDIL has a new AAVLD-administered ring trial test for equine herpesvirus**.
- **Dr. Doris Miller won the 2013 UGA Walter B. Hill Award for Distinguished Achievement in Public Service and Outreach**, which “recognizes distinguished achievement in public service and outreach”.
- **Dr. Sree Rajeev** achieved board certification with the American College of Veterinary Pathologists (ACVP) in September 2013.

**NEW TEST IN TIFTON**

- **The brucellosis ring test** was implemented, which is an economical screening test for bovine brucellosis herd screening in bulk tank milk.

**NEW TEST IN ATHENS**

- **The Clinical Pathology section at the TVDIL has a new instrument that measures ionized calcium and ionized magnesium**. Cost is $15 per test. For more information please see the article “A new tool for monitoring blood calcium concentrations in dairy cattle” in this issue of the Newsletter.

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**A New Tool for Monitoring Blood Calcium Concentrations in Dairy Cattle**

By John K. Bernard, Animal and Dairy Science, UGA, Tifton Campus
Jejunal Hemorrhage Syndrome (JHS) is a syndrome of the intestinal tract of unknown etiology that affects both dairy and beef cattle, but the incidence is considerably higher in dairy cattle. The syndrome goes by multiple names including JHS, Intraluminal Intestinal Hemorrhage Syndrome, Bloody Gut, Hemorrhagic Bowel Syndrome and others. JHS has a peracute onset of clinical signs ranging from complete anorexia, abdominal distention and pain, to recumbency and sudden death.

Affected animals may show sudden, complete anorexia, a severe drop in milk production, abdominal distention, signs of colic and ileus, decreased fecal output, melena, diarrhea containing either frank blood or blood clots, vocalization, or sweating. Affected cows usually do not have fever, but often have ruminal stasis, profound depression, and pale mucous membranes and may have atrial fibrillation or dehydration.

If the cow is standing, percussion of the lower-right abdomen can elicit a pronounced fluid slosh due to the backup of fluid and gas behind blood clots that are obstructing the jejunum. In some cases, jejunal loops of small intestine may be palpated with left shift and hyperinflated ileum. Serum chemistry may reveal hypocalcemia, hypocholesteremia, metabolic alkalosis, hyperammonemia, hyperkalemia, and hyperglycemia. Hyperammonemia, hypercalcemia, high anion gap, hyperphosphatemia, hyperphosphatemia, and azotemia have also been reported. Increases in creatine kinase (CK), γ-glutamyl transferase (GGT), sorbitol dehydrogenase (SDH), and aspartate aminotransferase (AST) have also been detected. The frequency of this syndrome has been increasing in recent years and has a very high fatality rate (85-100%). Medical and surgical treatments to date have generally been unrewarding. The condition has been recognized in many regions of the United States and Canada.

JHS should be suspected in cows with a history and clinical signs of peracute onset of anorexia, a drop in milk production, distended abdomen, abdominal pain, and passage of melena or feces containing frank blood. Palpation of distended loops of small intestine rectally and the presence of hypercoloicemia, hypochromasia, and metabolic alkalosis are diagnostically helpful findings. In some cases, a definitive diagnosis can be ascertained only with an exploratory laparotomy or a postmortem examination. Due to the ubiquitous nature of C. perfringens type A, a positive fecal culture for this organism is not diagnostic. Differential diagnoses for JHS include salmonellosis, winter dysentery, abomasal ulceration and hemorrhage, right abomasal displacement, and insufflation, intestinal volvulus, intussusception, and acute peritonitis. Postmortem examination usually reveals segmental lesions localized to the jejunum (Figure 1) containing clotted blood. There is severe necrohemorrhagic enteritis (jejunitis) with intraluminal hemorrhage or blood clots (Figure 2). Some cases may develop intussusception immediately adjacent to the area of segmental hemorrhage and clotting, and some affected cows have fibrinous peritonitis. The most prominent histologic findings in surgically collected biopsy specimens are severe, segmental, submucosal hemorrhage and edema of the small intestine. This is often accompanied by a mixed inflammatory infiltrate. Gram-positive rods (Clostridia) are evident in biopsy specimens in some affected cows.

The exact cause of JHS is unknown and the etiology is likely multifactorial. Clostridium perfringens type A (beta toxin positive) has been suggested as a possible cause of the syndrome. The bacterium has been isolated from the lesions of clinical cases and feed samples. However, experimental inoculation of C. perfringens type A into the abomasum and proximal jejunum of dairy cows has failed to reproduce the syndrome. This alone is not enough to rule-out the possibility that C. perfringens type A is involved with JHS because it may be a multifactorial disease with other contributing factors. Clostridium perfringens type A is frequently cultured from affected animals (85%), but it has also been suggested that Aspergillus fumigatus, other fungi, or fungal toxins may be involved in some cases of JHS. A number of epidemiological associations have been identified over the last 10 years that appear to result in increased risk of JHS including increased stress, increased milk production, high carbohydrate diet, use of milk urea nitrogen in diet analysis, decrease in dietary fiber, poorly fermented ensiled feeds, use of bovine somatotropin (growth hormone), and the winter season.

There are two primary hypotheses for JHS pathogenesis. One suggests that JHS is similar to hemorrhagic enteritis caused by C. perfringens type C in fast-growing suckling calves, lambs, or piglets. C. perfringens type C multiplies rapidly and produces toxins under conditions of high carbohydrate and protein substrate availability. It is possible that this scenario arises in adult dairy cows in association with other contributory factors similar to those that predispose to rumen acidosis. A second hypothesis suggests that improperly fermented ensiled feeds (i.e., poor silo or bunker management) may allow the accumulation of harmful molds/fungi, clonal bacterial, or other harmful bacteria, as well as their potential toxins which are then ingested by the cow, leading to the syndrome of JHS.

In summary, JHS is a usually a peracute, highly fatal intestinal syndrome of unknown etiology that affects both dairy and beef cattle. JHS should be considered when there is a sudden death or when signs of abdominal pain and distention, bloody feces, and pale mucous membranes are found. Necropsy findings usually reveal hemorrhage, necrosis and inflammation of a segment of the jejunum, with large intraluminal blood clots. Diagnosis may be made from clinical signs, history, exploratory surgery, and necropsy findings. Bovine practitioners should be aware of the clinical signs and necropsy findings in order to diagnose JHS and assist in a better understanding of the pathogenesis of this syndrome. Greater awareness and better recognition of this syndrome should lead to better understanding of its pathogenesis and methods of prevention.

References:
Creating a Culture of Quality in the In-House Laboratory

By Melinda S. Camus, DVM, DACVP
Assistant Professor of Pathology & Quality Manager of the UGA Clinical Pathology Laboratory

ASVCP Quality and Laboratory Standards (QALS) Committee Chair

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uality is defined as "a high degree or standard of excellence". While everyone would like to have a "high quality" veterinary practice, many contributing factors, such as laboratory quality, are often unrecognized and, therefore, unaddressed. Accurate laboratory values are crucial in making correct diagnoses and appropriate steps to ensure such results must be taken. In 2014, NAVTA (National Association of Veterinary Technicians in America) is certifying its first class of veterinary technicians in the specialty of clinical pathology. This demonstrates the emergence of clinical pathology as an important specialty discipline that requires a degree of expertise. Technicians with this certification may be of value in clinical practice. Additional details on the Academy of Clinical Pathology Technicians, which certifies these individuals, can be found at http://www.avcpt.net/.

In-house laboratory equipment varies greatly and can include handheld instruments (e.g. glucometers) and larger benchtop analyzers for all aspects of testing. The American Society of Veterinary Clinical Pathology (ASVCP) Quality and Laboratory Standard (QALS) committee recently published guidelines for point-of-care (POCT) testing in veterinary medicine. These quality recommendations for in-house laboratories, which have been summarized here, can be found in their entirety both in the December 2013 issue of Veterinary Clinical Pathology and online on the ASVCP website at http://www.asvcp.org/pubs/qas/index.cfm. To begin, all hospitals performing laboratory testing should have a written quality plan, which details standard operating procedures.

• Internal instrument QC refers to QC functions incorporated directly into analyzers. These provide important information and should be run as indicated by the instrument manufacturer. While internal QC assesses instrument function, only limited programs evaluate reagents and operator proficiency.

• Analysis of control materials refers to measuring standards with known analyte concentrations/activities. Use of such materials allows for evaluation of the entire POCT system, including instrument, reagent, and operator. While analysis of multiple levels of control (high, normal, low) for each analyte is ideal, this is not always practical in a clinical setting and may be reserved for initial instrument set up and troubleshooting. A minimum of one level of control, using control materials derived using the same methodology as the instrument being tested, should be performed each day of testing.

• External quality assurance (“proficiency testing”) refers to participation in an external quality program to which individual hospital laboratories subscribe. Materials for analysis are sent to all participating laboratories by the program manager and results are compiled. This allows for each individual lab to compare its performance with that of other laboratories using the same or very similar methodology. Quarterly participation is advised. The ASVCP QALS committee has prepared guidelines for participation in such programs, including a list of providers, with an updated publication in 2014.

• Comparability testing involves sending an aliquot of a patient’s sample to a reference laboratory to “recheck” or “confirm” results obtained on an in-office analyzer. This type of testing is often done as needed when an unexpected or aberrant patient result is derived from an in-house analyzer.

Utilization of these quantitative QC methods is imperative to a laboratory quality system. However, data derived from such assessments is only valuable if it is periodically reviewed and associated problems are specifically addressed. For example, it is not enough simply to run control samples periodically; they must be “in.” Periodic review of comparison results may reveal trends (e.g. control values are “in” but are creeping higher and higher) that point to impending instrument malfunction or failure (e.g. a lamp outage) that may be avoided with more immediate attention.

Developing a QC program is a daunting task and requires one or more individuals committed to nurturing its success. Consultation with a clinical pathologist with knowledge in this area may be of value when designing and implementing such a program in order to determine the appropriate type of control material, testing frequency, etc. Devising and enforcing a sample rejection policy is also key, particularly in hematology, where improperly filled tubes, unlabeled and/or clotted specimens, which are unfit to test, are redawn prior to analysis.

In addition to the quantitative QC measures discussed previously, nonstatistical QC methods should be employed, particularly with regards to hematology. Even the most advanced hematology analyzers utilize reference laboratories that cannot adequately assess cell morphology. Thus, manual review of blood smears to detect toxic change, aggregation, the presence of immature cells in circulation (e.g. band neutrophils, nucleated erythrocytes), and the presence of parasites is necessary. While it is likely time and cost prohibitive to evaluate a blood smear for each in-house CBC, a minimum a blood smear should be reviewed for CBC’s from all ill patients and those on which results are unexpected.

• Review should be performed by a qualified individual (e.g. veterinarian, veterinary technician, or clinical pathologist) and results of each review should be recorded.

Quality laboratory results are essential to making good clinical decisions in order to correctly diagnose, monitor, and treat all patients. Incorrect data, which can result in an incorrect diagnosis, is worse than no data at all. Ensuring the accuracy of such information is an aspect of running a hospital that is often forgotten or ignored; yet it is critically important.

Resources are available to assist in the design and implementation of a quality program at all levels of veterinary practice. Incorporation of such a program into the daily operation of a veterinary hospital will enhance the overall culture of quality, helping practices reach the “high quality” standard which they all strive to attain.


Rabies Submissions

Rabies infection occurs when an infected animal bites another animal or human and the rabies virus is transmitted via the infected animal’s saliva. Rarely, rabies is spread when infectious material, such as saliva, from a rabid animal comes into contact with mucous membranes, such as the eyes, nose, or mouth, or a wound.

The diagnostic laboratories in Tifton and Athens use the Georgia Public Health Laboratory (GPHL) in Decatur as a referral lab for rabies testing when a human or domestic animal exposure to rabies is suspected. The GPHL charges a $145 rabies testing fee, but the fee is waived when testing is related to a human or domestic animal exposure. However, to qualify for the fee waiver, all cases have to be submitted through the State Electronic Notifiable Disease Surveillance System (SENDSS), an online information database used by the GPHL, County Health Environmental Division, and submitters to exchange important case-related information.

To submit to our laboratories an animal head or entire carcass for rabies testing that involves a human or domestic animal exposure, follow federal regulations for packaging and shipment of infectious materials and use the following two steps:

1. First, report the exposure incident to your county Environmental Health Division or Animal Control. The county will generate a bite incident number in the SENDSS database.

2. Ship the specimens to the lab, along with the SENDSS number and our submission form. Testing and results reporting will be delayed if specimens are received in the laboratories without a SENDSS number.

In order to report a rabies exposure incident to your county officials, have the following information handy:

• All contact information (including dates of birth and county of residence) for any people involved in the incident. All efforts must be made to collect animal owner and any bite victim information before submitting the specimen.

• All available information for the attacking or animal, including the county in which the incident occurred.

• A thorough description of the incident or animal history. In addition to forwarding your samples to the GPHL for testing, the Athens Laboratory can also conduct in-house rabies testing on cases without documented human or domestic animal exposure, at a fee of $15. Note that submissions received by the diagnostic laboratories on Fridays will be shipped to the GPHL on the next business day.
The Serology/Virology staff at the TVDIL includes one laboratory manager, 3 full time technicians and a part time technician. Ms. Michele Coarsey, the laboratory manager, coordinates day to day activities of the section and has been with TVDIL for 17 years. She has a MS in Animal Genetics, is a registered Veterinary Technician (RVT) and holds a Specialist in Virology certification (ASCP). Ms. Deborah Blakey, Serology Technician III, has been with TVDIL for 25 years and she has a BA in Biology and Music. Ms. Candice Jackson, Technician III has been with TVDIL for 5.5 years and has a BS in Biology. She is in charge of NAHLN related PCR testing. Ms. Karina Sorensen has been with TVDIL for 1 year as a full time Virology Technician II and holds a BS in Biology. She was a part-time student worker with the TVDIL and was recognized as one of the top 100 student workers for UGA while in that position. Mrs. Katy Jones has been in the Section for almost a year as a part time technician and has a BS in Animal Science and was previously a registered Equine Medical Technician. Serology Virology Team is led by Dr. Sree Rajeev who is board certified Veterinary Microbiologist. The section specializes in diagnosis of viral diseases in a wide range of animals. This section also performs surveillance testing for brucellosis and pseudorabies. The section utilizes many different test methods to diagnose animal diseases and constantly tries to improve existing tests to better assist our clients. We strive to serve with excellence and are eager to discuss with clients how we can best suit their needs.

The administrative and support staff section at the AVDL is one of the functionally most diverse groups and includes some of the lab’s most long standing employees. Joanne Greenway (Administrative Assistant and proud Canadian) is our ever cheerful receptionist and special events planner and has been with the lab since 2011. Amy Lavender (Accountant) has been with the lab since 2007 and has been in her current position since 2011. She is also the busy mom of a teenage cheerleader, band member, and budding scientist. Wendy Counkle and Jennifer Lightsey (both Administrative Associates employed by the lab for 11 and 5 years, respectively) are collectively known as “Wendifer”. They receive, unpack, process, and distribute the numerous samples received by the lab each day in addition to fielding and routing phone calls. Jennifer is an avid reader and Wendy is a proud owner, breeder, and defender of the much maligned American Pit-Bull terrier. Shirley Smith (Administrative Assistant since 2006), the proud mom of a talented guitarist, maintains scanned records and keeps our slide file in excellent order in addition to assisting in accessions and answering phones as needed. Sabrina Bailey (Administrative Associate and the Purple Queen) has been with the lab since 1992. She oversees the accessions area but is also a jack of many trades, assisting with several other varied tasks around the lab. She is unfailingly cheerful except when having her picture taken.

The best way to find yourself is to lose yourself in the service of others. – Mahatma Gandhi