Welcome to the Fall 2013 issue of Diagnostic Veterinary Matters! It’s been a busy summer for us as we continue to work through the challenges of reduced state and federal funding while simultaneously expanding and improving the services we provide veterinarians and animal owners throughout the state. We continue to work with stakeholders, the Department of Agriculture, legislators, UGA senior administration, and the Governor’s Office of Planning and Budget to address the funding issues. At this point we believe we can stabilize the GVDLS budget situation with a combination of an equipment bond to replace old, outdated equipment and a modest increase in the FY2015 operating budget. We appreciate very much the continued support provided by numerous stakeholder groups and individuals as we address our budget difficulties. A number of facility upgrades have been completed primarily at the TVDIL including the conversion of the HVAC system and incinerator to natural gas from heating oil. This upgrade has already significantly reduced fuel and other overhead costs. Planning for replacing the roof of the TVDIL is underway as well. The AVDL has recently migrated to VetView, our new diagnostic laboratory information system, which will address problems inherent in the old system and offer new features that will enhance laboratory productivity and provide us opportunities for improving services. The TVDIL will migrate to VetView beginning in FY15. We continue to make improvements to our website in an effort to improve ease of use by our clients and to more effectively disseminate information. The GVDLS is exploring new ways to expand and improve our diagnostic services by evaluating the feasibility of a courier service in the Atlanta-Athens area and the use of biopsy sample submission kits at both labs. In addition, the GVDLS is bringing online a number of new diagnostic tests. We have developed an Eastern Equine Encephalitis (EEE) immunohistochemistry test that will provide more timely diagnosis than virus isolation and less regulatory problems since EEE is currently listed as a select agent by the Center of Disease Control (CDC). Based on feedback from producers and veterinarians we have developed a new Bovine Virus Diarrhea (BVD) testing strategy (see article inside) that will reduce client costs for herd testing, with an associated decrease in turnaround time. Finally, with the recent expansion of the deadly Porcine Epidemic Disease outbreak to neighboring North Carolina, the GVDLS has developed and validated a PCR assay (see article inside) in the event this disease enters the Georgia swine population.

The GVDLS is striving to provide you with the most complete, highest quality and timely services that are possible. We want to thank all our clients for your business and your continued loyalty to the GVDLS; we look forward to continuing to provide you with the best diagnostic services for the future.

Murray E. Hines II
Jeremiah (Jerry) T. Saliki
The Athens Veterinary Diagnostic Laboratory recently played a key role in identifying morbillivirus as the cause of the unusual mortality event that has caused the stranding of hundreds (about 500 as of this writing) bottlenose dolphins in the Mid-Atlantic Region from New York to North Carolina. This activity shows how tests systems developed for domestic animals can be leveraged for monitoring and improving the health of other animal species of national importance.

Laura Griffiths and Rachel Steffens successfully completed the 2013 pseudorabies ELISA gI and gB tests with perfect scores.

Ingrid Fernandez and Sarah Bates successfully completed the 2013 proficiency tests for avian influenza, New Castle disease, and swine influenza, achieving 100% scores in all three tests.

Jennifer McClain and Gulnaz Saikh successfully completed the 2013 BSE (mad cow disease) proficiency test.

The Serology staff successfully completed the 2013 Coggins, bluetongue, and bovine leukemia AGID proficiency tests, achieving 100% scores in all three tests.

The Serology staff successfully completed the 2013 Johne’s disease ELISA proficiency test with a 100% score.

The Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) has diagnosed a total of 17 cases of eastern equine encephalitis (EEE) this summer. All cases were from horses except one which originated from a dog with neurological signs. EEE is a mosquito-borne viral disease prevalent in eastern United States which causes serious disease in horses, man and birds. Clinical signs in horses include fever, anorexia, depression, hyperexcitability, blindness, ataxia, recumbency convulsions and death. A serum IgM ELISA (for equines only) and hemagglutination inhibition test (for all species) is available at TVDIL for confirmation. Mosquito control and vaccination of horses is highly recommended to prevent this disease. For more information on human infections visit http://www.cdc.gov/ncidod/dvbid/arbor/.

Shabrieka Farley as our new histology technician. Shabrieka lives in Sylvester and has a baby girl. She graduated from Darton College in Albany with an Associate Degree in Histology Technology. She has a HT certification from ASCP.

We welcome Katy Jones who has recently joined the TVDIL team as the front desk receptionist for the afternoons.

The TVDIL Virology/Serology section welcomes Karina Sorensen as our new laboratory technician and Rebecca Grantham as our new student worker.

Michele Coarsey, Deborah Blakey, and Candice Jackson passed Pseudorabies virus (PRV) gB and gI ELISA proficiency tests.

Michele Coarsey, Deborah Blakey, and Candice Jackson passed Brucellosis Buffered Acidified Plate Test (BAPA) and card proficiency tests.

Jill Johnson passed Johne’s disease liquid culture and pooled culture proficiency tests.

Candice Jackson and Jill Johnson passed Swine Influenza Virus NAHLN RT-PCR proficiency test.

Candice Jackson, Michelle Coarsey, and Jill Johnson passed Avian Influenza and Newcastle Disease NAHLN RT-PCR proficiency test.

Serology staff passed Equine Infectious Anemia (EIA) AGID proficiency test. Bovine Leukosis (BLV) proficiency test, Bluetongue (BTV) proficiency test, Johne’s disease ELISA proficiency test.

We now offer deeply discounted testing for BVD persistently infected animals using both the ELISA and PCR pooling techniques (see page 6).

We offer new PCR tests for Feline leukemia virus and Feline immunodeficiency virus. Cost is $32 / test.

The Clinical Pathology section of the TVDIL offers a variety of Endocrinology tests including T4, FT4, T3, FT3, TSH, Cortisol, Estradiol, Testosterone, Progesterone, Dexamethasone, and Insulin. We also offer special chemistry tests including Blood Ammonia, Phenobarbital, Fructosamine (Glycated Serum Protein Assay by DIAZYME Labs), Bile Acid, Iron, UIBC, Urine chemistries, and Cholinesterase.

We are in the process of getting a new instrument that can perform ionized Ca and ionized Mg. We anticipate these tests to be available by mid Fall. With this instrument we will be able to also implement other tests upon demand (for instance blood gases).
Fungal Cytology
By Bridget C. Garner, DVM, PhD, Dip ACVP (Clinical Pathology)
Department of Pathology, UGA CVM

Fungal agents can be seen in aspirates and impression smears of the skin, lungs, lymph nodes, bone and other organs. Yeasts may even be detected in bronchoalveolar lavage and cerebrospinal fluids, although this is less common. Below is a brief summary of a few of the fungi that can be recognized in cytologic preparations.

**Blastomyces dermatitidis**
Blastomycosis is typically seen in dogs, but it can be seen in other species such as cats. The disease is endemic in the Midwest and areas near the Mississippi and Ohio River basins, but cases are often seen in the southeastern U.S. as well. Cytology samples will contain large numbers of neutrophils, macrophages, multinucleated giant cells and lymphocytes as well as some spindle-shaped cells. The yeasts, which may be few to numerous in the cytologic preparations, are approximately the same size as a neutrophil (7-10 µm) and have a refractile, deeply basophilic, thick cell wall. Most organisms are single, but broad-based budding may be visible. These organisms are larger than *Histoplasma capsulatum*, smaller than *Coccidioides immitis* and lack the thick, clear capsule of *Cryptococcus neoformans*.

**Cryptococcus neoformans**
Cryptococcosis occurs in many geographic areas including Georgia. Both dogs and cats can be infected, and the upper respiratory and central nervous systems are more commonly involved than others. Inflammation is often minimal, but when present it is usually granulomatous with a predominance of macrophages. In some cytologic preparations, *Cryptococcus* organisms may outnumber inflammatory cells. The granular blue yeasts are round to oval and often surrounded by a thick, clear capsule although occasionally the capsule will be absent. The diameter of this yeast is more variable than *Blastomyces*. The organism’s diameter 40 µm if the capsule is present. This yeast divides by narrow-based budding, not broad-based budding like *Blastomyces* spp.

**Histoplasma capsulatum**
Histoplasmosis has a similar geographic distribution to blastomycosis, but cases can rarely be seen elsewhere. The skin and/or gastrointestinal tract of dogs are often affected. Numerous small (2-4 µm; approximately ½ the size of a RBC) oval yeasts are often scattered among neutrophils and macrophages, and the yeasts can be found within the inflammatory cells as well. These yeasts have a thin, clear halo and a crescent shaped pink to purple staining nucleus. *Histoplasma capsulatum* does cytologically resemble *Sporothrix* sp. (not shown), but *Sporothrix* sp. is more cigar-shaped whereas *H. capsulatum* has more round to oval borders.

**Coccidioides immitis**
This agent is endemic to the southwestern U.S. and is not usually seen in this region unless an individual has traveled recently. The lungs and bones of dogs are generally involved. Pyogranulomatous to granulomatous inflammation may be present. Low numbers of thick-walled spherules that are very large (measuring 20 to 200 µm in diameter) can be seen, and they contain small round endospores (2 to 5 µm) that may also be free in the background if a spherule has ruptured. The large size and the presence of endospores are the most unique and distinguishing features of *C. immitis*.
What is bVD?

- Bovine viral diarrhea is a significant cause of economic losses (estimated at $50-$100 per cow during an outbreak) to the beef and dairy industries. Clinical signs include: reproductive failure (failure to conceive, embryonic loss, abortion, congenital defects and stillborn or weak unthrifty calves) and respiratory disease or diarrhea.
- High proportions (about 70%) of infected cattle do not become sick. However, the virus may suppress the immune system and make them more susceptible to other diseases. Moreover, some asymptomatic animals are actually very dangerous because they may be carriers of the virus for life, also known as persistently infected, or PIs.
- PI animals result from calves infected with certain strains of the virus (non-cytopathic strains) in the womb. Although many PI calves are born unthrifty and die or are culled within 6 months, some become adults. Such PI animals shed large amounts of the virus in all secretions (saliva, feces, urine, milk, etc.) throughout their lifetime and continuously expose other members of the herd to BVD virus.
- Transmission of the virus from PI’s to susceptible herd mates can be by direct contact (e.g. nose to nose) or through contaminated veterinary equipment (e.g. needle or castrating and dehorning tools), farm equipment or facilities.
- Management practices to control BVD include vaccination of all members of the herd (starting at 2-3 months and 4-5 months old for dairy and beef calves, respectively), biosecurity procedures to prevent introduction (e.g. isolation and confirmation of BVD negative status of new additions and avoidance of pregnant additions), bio-containment to interrupt the spread of the virus, and laboratory testing of samples for the presence of the virus in a herd, with the final goal of identifying and removing PI animals.

Laboratory testing:

There are three situations in which laboratory testing is needed:

1. Diagnosis: an animal is sick or has aborted and you want to find out if BVDV is the cause.
2. Herd screening: you want to test an entire herd to screen for PIs.
3. Animal marketing: you want to buy or sell a calf and you want to establish that it is not BVD-PI. If you purchase BVD-free bred heifers, you must still test the calf after it is born to assure you will not accidentally introduce BVD into the herd.

Samples to submit:

- From a sick animal: about 8 ml of whole blood in EDTA (purple top tube)
- From dead animal or aborted fetus: lung, spleen, lymph nodes, kidney or entire fetus.
- For herd screening or marketing: one ear-notch sample in a screw-capped tube or blood in a red top tube.

Testing strategy for PIs: The following table shows the most common tests, prices, and turn-around time. For large herds, we can pool up to 24 ear-notch or blood samples and run one PCR test in order to reduce your cost/animal, with a turn-around time of 2-3 business days. If any pool is positive, we will re-test all members of that pool using the Antigen Capture ELISA (ACE) test, at an additional cost and increased turn-around time. If time is of essence, we suggest that you request the ACE test upfront.

### bVD Testing at the Georgia Veterinary Diagnostic Laboratories - Athens and Tifton

**One Complex Disease - One Simple Sample - One Simple Strategy!!**

<table>
<thead>
<tr>
<th># of samples received at once</th>
<th>PCR pool size</th>
<th>PCR cost per head</th>
<th>Time to results (PCR)</th>
<th>ACE cost per head</th>
<th>Time to results (ACE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>NA</td>
<td>NA</td>
<td>$5.00 plus $10 Setup fee</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>5-23</td>
<td>NA</td>
<td>NA</td>
<td>$4.30</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>24 or more</td>
<td>24</td>
<td>$2.5*</td>
<td>2-3 business days</td>
<td>$3.30</td>
<td>24 hours</td>
</tr>
</tbody>
</table>

Notes: Actual cost of the PCR pool is $60. If a pool yields a positive result all 24 samples will be re-tested individually by ACE, taking the final unit cost to $60/head and adding one day to the turn-around time. Assuming a 0.5-1% BVD prevalence it is not cost effective for the producer to do the PCR pool of less than 96 animals are tested.

**Emergence of Porcine Epidemic Diarrhea (PED) Virus in the United States in 2013**

By Jeremiah T. Saliki, DVM, PhD, DACVIM (ADVL)

**Introduction**

In April and May 2013, a disease outbreak characterized by diarrhea and vomiting occurred in multiple mid-western American pig farms almost simultaneously. The clinical signs led clinicians and diagnosticians to suspect transmissible gastroenteritis virus (TGEV), a member of the Coronavirus family. Testing of fecal samples from sick piglets for TGEV and porcine respiratory coronavirus (PRCV) using standard laboratory techniques failed to confirm coronavirus. Interestingly, examination of the same fecal samples under the electron microscope detected coronavirus particles. The unexpected discovery of coronavirus particles from fecal samples that were negative for the two swine coronaviruses known to occur in the US (TGEV and PRCV) prompted further testing at Iowa State University and also at the Federal laboratory at Ames for exotic coronaviruses. These efforts resulted in the confirmation of Porcine Epidemic Diarrhea Virus (PEDV) for the first time in the USA.

**Geographic distribution, transmission and clinical signs**

Sporadic outbreaks of porcine epidemic diarrhea (PED) were first identified in the UK in 1971 and this infection has become endemic in most Asian countries since 1982. There was one incursion into Canada in 1980, but it has not been reported in North America since then. Coronavirus are highly contagious, and the outbreaks in the US spread rapidly. Within 6 weeks the virus had been confirmed in about 200 hog facilities in 14 states including Arkansas, Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, North Carolina, New York, Ohio, Oklahoma, Pennsylvania, and South Dakota. The virus is transmitted by the oral-fecal route, with an incubation period of 3-4 days. Contaminated personnel, equipment, or other fomites may introduce PEDV into a susceptible herd. The disease is clinically indistinguishable from TGEV, with the main clinical sign being watery feces that may be flocculent and foamy. Vomiting may occur and dehydration is a secondary sign. Morbidity and mortality can surpass 90% in suckling piglets less than 14 days old, but gradually decreases with age. Lesions at necropsy show enteritis with histological blunting of the villi involving the whole length of the small intestine.

**Diagnosis**

The most useful diagnostic specimen is feces or a loop of small intestine. A variety of diagnostic tests (including negative-staining electron microscopy, fluorescent antibody staining, electron microscopy, immunohistochemistry, or viral isolation) commonly used for TGEV diagnosis are available in our laboratory for presumptive PEDV diagnosis. However, PCR is the only technique that can differentiate between TGEV and PEDV. We offer a PCR technique that can detect both viruses; a positive result is followed by an additional test (sequencing) for definitive diagnosis.

**PED and Georgia**

The rapid spread of PED across the US and its occurrence in neighboring North Carolina raise concern for its possible introduction into Georgia. Fortunately, we are poised to rapidly detect the virus if it were to enter the state. The vigilance of swine practitioners and animal owners is key to detecting its incursion into our state. We recommend that PEDV testing should be requested for all cases in which an acute TGE-like disease is observed with high morbidity.


**GENERAL NOTES FROM THE LABS:**

- Both the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) and the Athens Veterinary Diagnostic Laboratory are operating as a full-service diagnostic laboratory branches of the Georgia Veterinary Diagnostic Laboratories. We offer complete necropsy of large, small and exotic animals as well as laboratory testing in Virology, Serology, Bacteriology, Mycology, Pathology and Clinical Pathology.
- For a complete list of all tests available and submission form, please visit our website [http://www.vet.uga.edu/dlab](http://www.vet.uga.edu/dlab).
- The labs currently offer Global vet link.
- We have had several packages sent to us via the United States Postal Service without sufficient postage. If you have a postage meter in your clinic, please check the calibration on your meter to ensure enough postage is being placed on packages.
- Remember – UPS is now our pre-paid courier service – if you still have any pre-paid FedEx labels in your clinic please DO NOT USE THEM. We will have to bill your clinic the full shipping costs incurred.
- We are able to email your monthly statements. If you would like your statements sent via email please let us know by dropping an email to: TVDIL_kgosims@uga.edu or rhhall@uga.edu.
- Please remember when sending diagnostic samples to the Tifton Lab through the U.S. Postal Service to use P.O. Box 1389 instead of the street address.
The Bacteriology and Mycology staff at the TVDIL includes one laboratory manager, three full time technicians and a part time laboratory assistant. This section has a combined total of over 107 years of experience at the TVDIL. Ms. Cindy Watson, the laboratory manager, has been serving TVDIL for 31 years and coordinates day to day activities of the lab. She has a BS in Microbiology, MS in Medical Microbiology, and is a Registered Microbiologist. Ms. Gail Clifton (Lab. Tech III) has a BS in Medical Technology and MT (ASCP) and has 21 years of experience. Ms. Jill Johnson holds an AS degree in business (Lab. Tech II) and has 22 years of experience. Ms. Teri Register holds AS degree in Animal Science and Livestock Health and has 23 years of experience. Ms. Barbara Goins, our Laboratory Assistant has 10 years of experience. The bacteriology and Mycology team is led by Dr. Sree Rajeev who is a board certified Veterinary Microbiologist. Our lab is passionate about serving the animal industry to improve the health and wellbeing of the livestock and companion animals of the state of Georgia.

"THE REWARD FOR WORK WELL DONE IS THE OPPORTUNITY TO DO MORE"  
(J. Saulk)