Welcome to the Spring 2013 issue of Diagnostic Veterinary Matters. We wish to introduce to you our new editor, Dr. Marcia Ilha. Drs. Hines and Saliki will continue to serve as associate editors and Dr. Paula Krimer will continue to serve as assistant editor. Dr. Ilha is an assistant professor of pathology and board certified anatomic pathologist at the TVDIL since 2009. She obtained her DVM degree from the Federal University of Santa Maria (UFSM) in Brazil, and completed her MSc at UFMS, Brazil. She earned a graduate diploma in veterinary pathology from the University of Guelph, Canada, and completed an anatomic pathology residency at the University of Tennessee, Knoxville, before becoming a faculty member at the TVDIL.

In November 2012, the American Association of Veterinary Diagnostic Laboratories (AAVLD) audited both the Athens and Tifton Diagnostic Laboratories for AAVLD accreditation. Based on feedback from the auditors and comments from the AAVLD Accreditation Committee, the audit went very well and both laboratories will be receiving full AAVLD accreditation for the next 5 years (2013-2017). AAVLD accreditation is a very important accomplishment for our laboratories as it verifies our laboratories have a high degree of expertise and quality control for the diagnostic services we provide. In addition, AAVLD accreditation is required for participation in the National Animal Health Laboratory Network (NAHLN) that provides surveillance activities for foreign animal diseases. Both of our laboratories are members of the NAHLN system.

In our last five issues we have shared with you the problems we have been facing as a result of persistent steep state budget cuts in the face of rising operational costs. A brief update on the financial situation follows. A number of UGA Administrative officials, Georgia legislative officials and numerous stakeholder groups including Georgia Cattlemen’s Association, Farm Bureau, Georgia Dairy Producers, Georgia Federation of Saddle Clubs, GVMA, SGVMA and Southwest Georgia Chamber of Commerce (23 counties) have been advocating for an increase in state funding for the Georgia Veterinary Diagnostic Laboratory System (GVDLS). Recently the Governor’s proposed FY13 amended and FY14 budgets have been released and did not include additional state budget reductions. In addition, a small increase to cover annual employee benefits was included in each budget. Assuming that the Governor’s proposed allocations to our FY13 and FY14 budgets receive legislative approval, there will be no need to cut any services in the GVDLS. However, these adjustments will not solve all the financial issues of the GVDLS, and a $3-4 million equipment bond issue is direly needed in FY15 to replace old and outdated equipment at both D-Labs. The labs cannot afford to replace costly diagnostic instruments from our operating budget. If an equipment bond is not obtained in FY15, the GVDLS will slip back into financial difficulty. We wish to express our appreciation to the numerous stakeholder groups and individuals that have been and continue to be strong supporters of the GVDLS.

We remain committed to utilizing a combination of process improvement/increased efficiency methods, technological improvements, maximizing synergies between laboratories and the College of Veterinary Medicine, pursuing federal funding, expanding profitable services to the research and exotic animal/wildlife communities in order to continue maintaining two full-service diagnostic laboratories in Georgia. We want to thank all our clients for your business and your continued loyalty to the GVDLS, and look forward to continuing to provide you with high quality diagnostic services for the future.
Ashley Phillips, Amy McKinney, and Sarah Quattlebaum successfully completed the 2012 AAVLD Inter-Laboratory Bacteriology Quality Assurance Survey with 100% Accuracy.

Sarah Bates and Ingrid Fernandez successfully completed the 2012 FMD-CSF proficiency test.

Laura Griffiths, Pam Currin and Rachel Steffens successfully completed the 2012 individual Leptospirosis MAT proficiency test.

Ten staff and faculty members completed the USDA FY13 Security Rules of Behavior training, which is required for our NAHLN membership.

Melissa Gandy passed the American Society of Clinical Pathology (ASCP) Histology Technician exam in November of 2012. She is now an official certified histotechnician.

Candice Jackson, Debbie Blakey and Michele Farrar successfully completed the Johne’s ELISA proficiency test.

Candice Jackson and Michele Farrar successfully completed Lepto MAT proficiency test.

Ashley Burroughs, Candice Jackson, Michele Farrar and Jill Johnson successfully completed the FMD and CSF PCR proficiency tests.

The Serology staff successfully completed the Anaplasma cELISA proficiency test.

The Clinical Pathology staff successfully completed the Hematology, Chemistry and Urinalysis quarterly proficiency tests.

Serology -

We now offer two new serology profiles, with 1-2 day turn-around time:

- **Canine tick-borne disease panel** tests for Babesia canis, Bartonella henselae, Bartonella vinsonii, and Rickettsia rickettsii at a cost of $100;

- **Complete canine infectious disease panel** includes tests for canine heartworm, Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Ehrlichia canis, Ehrlichia ewingii, Babesia canis, Bartonella henselae, Bartonella vinsonii, and Rickettsia rickettsii, at a cost of $125.

Molecular Tests –

- **Combo test for Tritrichomonas** culture and PCR for $35. The culture must be submitted in a pouch. PCR can be performed on the same pouch sample after 5 days’ incubation or immediately on a freshly submitted preputial wash.

- **Bovine adult diarrhea panel** includes BVD, Salmonella, Johne’s, Coronavirus and Bovine enterovirus for a cost of $90.

From the clinical pathology laboratory:

**Reticulocytes** - are now being quantified using the automated hematology analyzer rather than using the manual method. This improves both accuracy and turn-around times. No changes should be noticed in reporting or in reference intervals.

**Ketones** - Confirmatory testing for the presence of ketones in urinalysis specimens is back! After months on manufacturer's backorder the reagents for confirmatory testing are available again. Confirmatory testing for urine bilirubin is still unavailable, with an anticipated arrival date of June 2013.
Evidence suggests that Bovine Virus Diarrhea Virus (BVDV) naturally occurs in free-ranging white-tailed deer (WTD, Odocoileus virginianus) in North America. BVDV has been isolated from WTD, and studies have suggested that infection, persistent infection (PI), and clinical disease in deer, and transmission between deer and cattle are possible as a result of experimental infection. However, the role of WTD as a reservoir is not fully understood. Previous studies have demonstrated the presence of BVDV in populations of free-ranging WTD in other states in the US. The occurrence of BVDV in these populations was very low (0.3% or less).

During the hunting season of 2010-2011, we collected ear notches from 367 hunter-harvested deer from 37 counties in Georgia (Figure 1). The age of the animals varied from 6 months to 6.5 years and 42% were under 2 years. Sex distribution was 48.5% female deer, 51% male deer, and 0.5% of unknown sex. Initial screening of the 367 samples for BVDV using AgELISA resulted in 364 negative samples and 3 suspect samples. The 3 suspect samples were negative for BVDV by PCR, virus isolation, and immunohistochemistry. A subpopulation of 89 samples selected from various geographical regions also tested negative for BVDV by PCR. Although a few of the samples were initially suspect for BVDV, the presence of the virus within the deer population studied could not be further confirmed.

Results from the current preliminary study may not support the hypothesis that WTD is a potential reservoir for BVDV in the State of Georgia. However, low prevalence of BVDV in populations of WTD in Georgia as seen in other states is still possible because BVDV infection has been observed in other species of deer in this state. In a previous study by other faculty at the TVDIL, a fallow deer (Dama dama) from Little St. Simons Island was positive for BVDV and was considered a PI animal.

Regarding the population of WTD studied, we believe that age and sex distribution has not influenced the results. Bovine Viral Diarrhea PI calves typically succumb to BVDV under the age of 2. Hunter-harvested deer populations are typically biased toward adult males; however the deer population represented in the current study was composed of 42% animals less than 2 years of age and 48.5% of females. The deer population in the State of Georgia is estimated to be approximately 1.2 million. Based on this estimate, approximately 0.03% of the deer population in Georgia was tested in the current study. Future studies targeting a larger population may be necessary to determine if BVDV is truly present in WTD in Georgia.
Cytology is a relatively inexpensive, rapid, and safe diagnostic technique for evaluating a variety of lesions. In many instances, a definitive diagnosis is possible. Despite the best technique in obtaining the specimen and making the smears, there are some lesions that cannot be diagnosed by cytology. In such cases, however, cytology can narrow the list of rule-outs for a lesion and suggest additional diagnostic tests that would be appropriate, e.g. biopsy and histopathology, culture, etc.

Q: Which lesions are most diagnosable by cytology?
A: Lesions composed of a single cell type are the most amenable to diagnosis by cytology. These are most notably round cell tumors (lymphosarcoma, mast cell tumor, histiocytoma, etc.) which are composed of a homogeneous neoplastic cell type and tissue architecture is irrelevant for diagnosis. Simple inflammatory lesions are also typically identifiable by cytology.

Q: How can cytology provide a partial diagnosis that requires additional testing?
A: When there is a combination of cell types, e.g. inflammatory cells + proliferating epithelial cells or mesenchymal cells, it is frequently not possible to determine which is the primary lesion and which is secondary. Whenever a tissue becomes inflamed, reparative changes include proliferation of connective tissue/mesenchymal cells and/or proliferation of epithelial cells. Rapidly proliferating cells, whether reactive or neoplastic, exhibit prominent variation in size and appearance which are cytologic features of malignancy. Consequently, examination of tissue architecture by histopathology is required to differentiate a primary inflammatory lesion with reparative proliferation of epithelial and/or mesenchymal cells from an epithelial or mesenchymal tumor with secondary inflammation.

Some masses can be identified as a specific cell type but it is not possible to determine whether the lesion is nodular hyperplasia, an adenoma, or a well differentiated malignancy. For example, sebaceous gland and perianal gland masses are typically identifiable by cytology but a definitive diagnosis is not possible without knowledge of the tissue architecture and surrounding tissues.

For some tumors, histopathology is necessary to determine the mitotic index which is required to determine the biologic behavior of the tumor. For example, when the tumor cells comprising a melanoma are pigmented and uniform in appearance, it is not possible to determine whether the tumor is benign or malignant. The mitotic index has been found to be the most accurate means of determining biologic behavior as benign or malignant. In such cases, histopathology is required because the mitotic index is determined by counting mitotic figures in 10 high power fields. Similarly, determination of the mitotic index of a mast cell tumor has been found

Cytology of an abscess with large numbers of degenerate neutrophils and both intracellular and extracellular bacteria.
Uses and limitations of Cytology as a Diagnostic Technique

Pauline M. Rakich, DVM, PhD, DACVP (AVDL)

Cytology of a cultured immortal cell line displaying marked malignant criteria including anisocytosis, anisokaryosis, and bizarre nuclei.

Q: Which lesions are the least likely to be diagnosed cytologically?
A: Lesions that are highly vascular, very firm or hard, mammary masses, and mesenchymal lesions are least likely to be able to be diagnosed by cytologic examination. Highly vascular lesions tend to yield large amounts of blood which can dilute any cells that comprise the lesion or may only yield blood and thus are nondiagnostic. Firm or hard lesions tend to contain large amounts of stroma such as collagen, cartilage, and/or bone and thus do not exfoliate cells sufficiently to be diagnostic. In contrast, although mammary masses readily exfoliate cells, they are usually not diagnostic because mammary tumors are typically composed of a mixture of cell types and they are commonly not homogeneous in that the tumor cells may vary greatly in their degree of differentiation with some portions of the tumor appearing appear benign while other portions of the tumor cells appearing malignant. Examination of the entire tumor and the surrounding tissue for evidence of local invasion is necessary for making a definitive diagnosis. All of the aforementioned lesions typically require biopsy and histopathology for diagnosis.

The most difficult lesions to diagnose cytologically are mesenchymal masses. Since connective tissue cells normally proliferate whenever there is tissue damage and because rapidly proliferating cells typically exhibit cytologic atypia, it is frequently not possible to differentiate reactive connective tissue from a connective tissue tumor. In histologic sections, reparative connective tissue is identified by gradual maturation with progressively decreased cellularity, more uniform cells, and increased amounts of stroma. These architectural features are lacking in connective tissue tumors; but since tissue architecture is not visible in cytologic specimens, cytology is frequently nondiagnostic in lesions consisting of proliferating connective tissue cells, except in the case of extremely anaplastic mesenchymal tumors. Diagnosis of mesenchymal lesions is sometimes not even possible histologically when the biopsy is too limited to be able to see the tissue architecture.

As long as the limitations of cytology are understood and discussed with the owner, cytology is a useful test for determining which lesions can be treated conservatively and which may need more aggressive diagnostic testing or treatment. Whenever there is a question regarding the suitability of cytology or the proper means of specimen preparation, consultation with a pathologist is encouraged to achieve the most diagnostic information possible.
Several dogs on a rural Georgia farm died approximately one week following a wild hog-hunting trip. One animal was submitted to the TVDIL with a history of self-trauma to the right side of the face and neck. At necropsy the right side of the face was swollen and erythematous with multiple excoriations. The right eyelids were edematous and swollen shut. Histologically, there was nonsuppurative meningoencephalitis involving the right side of the brainstem. Typical intranuclear herpesviral inclusions compatible with Pseudorabies virus (PRV) infection were observed within neurons.

Pseudorabies (Aujeszky’s disease) is caused by an enveloped DNA virus—an alpha herpesvirus. Pigs are the primary host, and because of adaptation, the disease is often subclinical in this species. Infection of companion animals occurs only in areas where the disease is enzootic in the pig population. Natural infection occurs after virus ingestion—either the consumption of infected meat or biting infected pigs. The virus enters nerve endings and travels through the nerve fibers to the brain. Incubation time in dogs and cats is 3 to 6 days. The virus generally produces severe clinical signs in dogs and cats, and the total course of the disease is seldom more than 48 hours. Initial signs include behavioral changes which range from inactivity and indifference to restlessness and aggression. The cardinal sign is intense pruritis which usually occurs in the head region and may be unilateral. Violent scratching and head rubbing may be observed. The resulting swelling, erythema, excoriations and skin ulcers are due to self-mutilation. Other neurologic signs (anisocoria, mydriasis, vocal changes, etc.) are attributed to cranial nerve dysfunction and may be unilateral. There are no typical hematologic or biochemical findings in pseudorabies. Diagnosis can be achieved through direct fluorescent antibody or PCR testing. Fresh brainstem and tonsil are the recommended specimens for testing. If the clinical signs are unilateral, sections of brain from the affected side should be submitted for testing.
The State of Georgia recently renegotiated their contract pricing with FedEx and UPS. As a result, the Athens and Tifton Laboratories can no longer offer FedEx preprinted labels as we are unable to pass on a discount to you. If you have FedEx preprinted labels you were previously sent, please discard them. If you are interested in using our discounted service with UPS, please call us!

REMINDERS

■ **Submission Forms** - To ensure accuracy, please make sure you are always using the latest version of our submission form and discard older versions. New forms can be found at [http://www.vet.uga.edu/dlab/](http://www.vet.uga.edu/dlab/).

■ **Client Communication** - Privacy policy regarding disclosing Information: We have had an increase in animal owners calling for information. When this happens, we always direct them to call your office. To maintain client confidentiality, we can only disclose/discuss test results with referring veterinarians or their designees. When calling in to check on results, please have the Doctor’s License Number or Accession Number available to help us verify we are speaking with your clinic.

■ **Slide Mailers Available** - For a nominal fee of $10 that can be added to your monthly invoice, we will ship your clinic an assortment of 50 recycled slide holders. If interested, please call us at 706-542-5568.
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MEET THE STAFF

“We sweat the small stuff!”

The Bacteriology Staff at the AVDL includes a laboratory manager, 3 full time technicians and 1 part-time laboratory assistant with over 35 years’ combined experience. Paula Bartlett, Amy McKinney, Brian Min, Susan Sanchez (Director), Ashley Phillips, and Sarah Quattelbaum.

“CLINICAL PATHOLOGY”

“We are here to serve you!”

The Clinical Pathology section full-time staff at TVDIL includes Anita Merrill and Tammie Vann. Anita is the Clinical Pathology laboratory manager/section chief and holds the degree of Bachelor of Science (BS) from Berry College. She is a registered Medical Technologist (MT) by the American Society of Clinical Pathologists (ASCP). Tammie holds degrees of Associate of Science from Abraham Baldwin Agricultural College, BS from Georgia State University, and BS in Medical Technology and Laboratory Technology from Auburn University. She is a registered MT (ASCP). Anita and Tammie have been employed at the TVDIL for over 33 and 29 years, respectively. They are responsible for Hematology, Clinical Chemistry, Endocrinology, Urinalysis and Parasitology analyses. With over 60 years of combined experience, Anita and Tammie are highly qualified and knowledgeable technicians willing to provide the best service possible to our clients. Their goal is to produce high quality results with same day turnaround time.