Contents

01 TVDIL New Director
02 Feline Infectious Peritonitis
03 Clinical Flow Cytometry
05 MALDI-TOF Technology Update

Diagnostic Veterinary Matters
Great service, Trusted results.

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A native of Mumbai, India, Dr. Naikare earned his professional veterinary degree (BVSc) and a Master’s degree in veterinary microbiology (MVSc) from Bombay Veterinary College, India. Dr. Naikare received his PhD in molecular microbiology from Oklahoma State University and is a Diplomate of the American College of Veterinary Microbiologists. During his time at the TVMDL–Amarillo, he developed and introduced more than 15 new tests including PCR tests for *Tritrichomonas foetus*, calf diarrhea panel, viral bovine respiratory disease panel, *Mycoplasma bovis*, Malignant catarrhal fever, *Anaplasma marginale* and Cache valley virus. He was responsible for maintaining the TVMDL–Amarillo’s certification with the National Veterinary Services Laboratory for proficiency testing of highly consequential food animal pathogens and foreign animal diseases including Brucellosis, Johne’s disease, Avian Influenza, exotic Newcastle disease, and classical swine fever. In addition to his contributions nationally, he has consulted for the USDA Foreign Agriculture Service, creating and conducting training workshops on laboratory diagnostics for veterinarians in Vietnam and Honduras.

In addition to his experience in laboratory diagnostics, Dr. Naikare earned his MBA from West Texas A&M University in 2014 with a specialization in marketing that helped him hone a customer service focus and apply business concepts to laboratory processes. He participated in the Texas A&M AgriLife Advanced Leadership Program to focus on building relations with different agricultural agencies, state legislature representatives, federal employees, and key stakeholders in the Amarillo lab. He has served in leadership roles for a number of professional organizations including memberships on the Executive Board of the American Association of Veterinary Laboratory Diagnosticians, the Board of Governors of the American College of Veterinary Microbiologists, and the Editorial Board of the Journal of Veterinary Diagnostic Investigation.

Dr. Naikare’s combination of laboratory experience and business training give him an excellent understanding of the opportunities for growth in the Tifton area. Competition from private laboratories, reductions in federal and state support, and increased animal-side point of care diagnostics are all challenges that need to be addressed for the Tifton laboratory to continue to fulfill its missions of disease surveillance and serving the needs of veterinarians.
and food producers in our state. Dr. Naikare’s vision for the laboratory is to be a customer-centric laboratory that offers high quality, accurate, rapid and cost-effective diagnostic services for veterinarians in Georgia and beyond. Dr. Naikare hopes to improve services by focusing on improved communication with clients including field visits to local operations, fostering relationships with stakeholders, reviewing and reducing operational costs, and applying new technologies to make better diagnoses.

Dr. Naikare’s wife, Dr. Nikita Mirajkar, is a veterinarian and a neuro-toxicologist with a great passion for teaching. Their family includes two girls, six-year-old Riya and two-year-old Ritu. They enjoy traveling to scenic places, visiting parks, meeting new people, and experiencing diverse cultures. They are excited about moving to the warmth of Georgia which will allow immense opportunities for outdoor recreational activities.

In the Fall of 2016, the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) received a 7-month old, female, domestic short hair cat for necropsy. The animal presented to the referring veterinarian for general malaise and chronic weight loss. The cat returned home with the owner and was subsequently found dead a few days later. At necropsy, the animal had a decrease in body condition with mild generalized muscle atrophy. Following exposure of the abdomen, the mesenteric lymph nodes were markedly enlarged and both the liver and kidneys were expanded by numerous, variable diameter (1 to 30 mm), nodular, light beige masses (Figure 1). Differentials for the gross findings were a disseminated infectious process such as feline infectious peritonitis (FIP) or possibly lymphoma. Tissue samples from major organ systems were submitted for histologic processing. Histopathology of the gross kidney and liver lesions revealed a severe pyogranulomatous hepatitis (Figure 2) and nephritis; along with a pyogranulomatous pneumonia, lymphadenitis, meningoencephalitis, and myocarditis. Also, the affected tissues exhibited a concurrent vasculitis. The pattern of multisystemic pyogranulomatous inflammation with vasculitis was consistent with the noneffusive form of FIP. PCR on fresh samples of lung, liver, kidney, and brain were positive for Feline Coronavirus. These results in conjunction with the histopathologic lesions confirmed FIP in this animal.
FIP is a fairly common infectious disease of cats with a low morbidity, yet high mortality. The causative agent is Feline Coronavirus and more specifically, the feline infectious peritonitis virus biotype. The exact pathogenesis of the viral infection and development of clinical disease is not fully elaborated, but the ability of the virus to replicate within macrophages is important in the development of FIP. Other factors contributing to FIP include viral strain, genetic predisposition, and the host’s immune response (e.g. ineffective cell mediated immunity of the host will favour an FIP virus infection).

FIP manifests itself in two forms: the noneffusive (“dry”) form as displayed in this case and the effusive (“wet”) form. Despite the distinct nomenclature, these forms likely represent the two extremes of a spectrum. The effusive form typically presents with multiple acutely developing cavitary effusions (for example abdominal, pleural, and pericardial effusions). The noneffusive form however is chronic and characterized by multisystemic pyogranulomatous inflammation with vasculitis. The lesions are commonly observed in the kidney, eye, brain, lung, liver, lymph nodes, and serosal surfaces. Grossly, this form of FIP can present similar to systemic bacterial, fungal or protozoal (i.e. toxoplasmosis) infections along with disseminated neoplasia, especially lymphoma.

The diagnosis of FIP in cats can be difficult. Its presentation is similar to a variety of other diseases and a single definitive diagnostic test does not exist for FIP. Histopathology in combination with molecular diagnostics or immunohistochemistry is considered the gold standard. Other ancillary tests (e.g. cytology, fluid analysis, serology) may help to support clinical suspicions, but in the end, an antemortem diagnosis of FIP can be difficult to achieve.

Currently at the TVDIL, Dr. Eman Anis, a post-doctoral researcher working with Dr. Rebecca Wilkes, is leading a study examining the role the host immune response plays in the viral infection and the development of FIP. Hopefully such research can help in the development of antemortem tests and effective treatment for the disease.

As a result, the TVDIL is encouraging veterinarians to submit blood and fecal samples from cats suspected of having FIP, especially with biopsy or necropsy submissions.

Boosting sample numbers will help strengthen the data and improve the study. Please contact Dr. Anis, Dr. Wilkes, or other faculty at the TVDIL if you have questions regarding sample submission for this study by calling (229) 386-3340.

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**Veterinary Clinical Flow Cytometry**

**Athens Diagnostic Lab Offers New Technology**

Drs. Jaime Tarigo and Tina Meichner

The Athens Veterinary Diagnostic Laboratory is now offering Canine Clinical Flow Cytometry. This assay includes a comprehensive panel of 11 antibodies to aid in the diagnosis and prognosis of canine lymphoma and leukemia.

**What is flow cytometry?**

Flow cytometry is a test that determines the immunophenotype of cells by recognizing normal and abnormal expression of cell surface markers on T cells (helper T cells, cytotoxic T cells), B cells, neutrophils, and monocytes.

**When is clinical flow cytometry most often used:**

1. To help distinguish between reactive and neoplastic lymphocytes
2. To determine if lymphoma/leukemia is of B or T cell origin and identify specific subtypes of B or T cell lymphoma/leukemia which will aid in prognosis (see table)
3. To differentiate between lymphoid and non-lymphoid (myeloid/monocytic) neoplasia

Clinical flow cytometry is used most often in combination with clinical history/presentation and cytology/blood smear examination for diagnosis of disease.

In some cases, additional diagnostics (PARR-PCR for antigen receptor rearrangement, histopathology, immunohistochemistry, immunocytochemistry) are also required.

**When might clinical flow cytometry be most helpful?**

1. Lymphadenopathy, organomegaly, or mediastinal mass with the following cytology results:
   - Confirmed or probable lymphoma composed of intermediate to large lymphocytes
   - Homogeneous expansion of small to intermediate lymphocytes
• Suspected lymphoma or thymoma (for mediastinal mass)
  Sample to submit: Fine needle aspirate of lymph node/enlarged organ/mass (see sample submission guidelines)

2. Abnormal CBC findings consisting of:
   • Peripheral lymphocytosis with increased numbers of small mature, intermediate, or immature lymphocytes
   • Presence of any immature cells/‘blasts’ with a normal or elevated white blood cell count
  Sample to submit: Peripheral blood (EDTA tube)

3. Abnormal bone marrow findings with the following cytology/CBC results:
   • Increased numbers of blasts or small/intermediate lymphocytes with leukopenia on CBC
  Sample to submit: Bone marrow (EDTA tube) for samples with leukopenia on CBC. Peripheral blood (EDTA tube) is preferred if lymphocytosis is present.

4. Body cavity effusion with the following cytology results:
   • High numbers of small/intermediate/OR large lymphocytes consistent with or concerning for lymphoma
  Sample to submit: Cavity fluid (EDTA tube and red top tube when possible- 0.5ml in each tube, see sample submission guidelines)

Sample collection and shipping guidelines:
• Collect samples and ship on the same day overnight for Monday through Friday delivery
• Keep samples refrigerated (do not freeze) until shipped
• Ship overnight with an ice pack
• Samples should be submitted to the Athens Veterinary Diagnostic Laboratory

Courier services may be available in your area
Contact AVDL (706) 542-5568 or visit ugavetlab.org for information

For additional information/questions email: vetclinflow@uga.edu

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Canine multicentric B cell lymphoma.
Prescapular lymph node cytology (left) revealed an expansion of intermediate to large lymphocytes consistent with lymphoma. Flow cytometry (scatterplot, right) identified >94% of the lymphocytes as B cells (CD21+) with retained MHCII expression. In general, multicentric B cell lymphoma has a better prognosis than multicentric T cell lymphoma in dogs. In addition, loss of MHCII expression would have resulted in a poorer prognosis for this dog.

Immunophenotype and prognosis of canine lymphoma determined by flow cytometry:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Flow Cytometry</th>
<th>Prognosis</th>
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</thead>
<tbody>
<tr>
<td>B-cell lymphoma</td>
<td>High MHC Class II</td>
<td>Better prognosis (MST* 314 days)¹</td>
</tr>
<tr>
<td>B-cell lymphoma</td>
<td>Low MHC Class II</td>
<td>More aggressive behavior (MST* 120 days)¹</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>High MHC Class II, CD45-</td>
<td>More indolent behavior (MST* 637 days)²</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>Low MHC Class II, CD45+ and CD4+</td>
<td>More aggressive behavior (MST* 159 days)³</td>
</tr>
</tbody>
</table>

Technology Update – Bacterial Identification

Matrix-Assisted Laser Desorption Ionization Mass Spectrophotometer: Time of Flight

MALDI-TOF MS

Dr. Susan Sanchez
Athens Veterinary Diagnostic Laboratory

Accurate and fast diagnosis is always our goal in the microbiology laboratory. The constant challenge is to meet this goal while dealing with hundreds of pathogens that have different growth rates and growth conditions. We must isolate the culprit, grow it in quantities sufficient for identification, and then perform antimicrobial susceptibilities to allow for the correct choice of treatment. These steps normally take at least 72 hours from the time of putting the sample in the growth media. The fundamental target for clinical microbiology innovation is how to speed up accurate results given the constraints of bacterial growth times. Any innovations must be cost-effective and should have enough internal checks and balances to guarantee adequate sensitivity and specificity of each test. When we deploy innovative diagnostic technologies for patients in the microbiology laboratory, we prefer tried and true technologies over those that are still in a developmental stage. Until recently, clinical microbiology advances have only relied on enhanced identification of the phenotypic characteristics of the isolated bacteria or fungi.

However, the introduction of the mass spectrophotometry (MS) technology, called Matrix-Assisted Laser Desorption Ionization Mass Spectrophotometer-Time of Flight (MALDI-TOF), has been a game changer and created new possibilities.

MS technology has been used by clinical chemists for the diagnosis of cancer and other disease biomarkers. The newer Matrix-Assisted Laser Desorption Ionization mass spectrometry, MALDI for short, was first used nearly 30 years ago; their inventors received the Nobel Prize in 2002. Over the years MALDI has evolved and revolutionized the clinical microbiology laboratory. Application of MALDI now allows for bacterial identification within minutes after a pure growth is identified, reducing the overall turn-around time by over 24 hours, with the added benefit of a significant reduction in labor and the elimination of expensive phenotypic tests.

So how does MALDI-TOF work? MS technology uses a “soft ionization” mechanism, where a low-mass organic compound (matrix) is mixed with the microorganism. The matrix-microorganism mixture is spotted on a metal target for analysis. The spot containing sample and matrix is irradiated for a short time using a laser beam. The irradiation ionizes the sample releasing ionized proteins, which can be measured by a mass analyzer. This captured data provides detailed information regarding the composition of the sample in the context of mass to charge ratios. To identify the bacteria in the sample, this protein ionization data is used to generate a spectrum for the particular organism that is compared to a database of unique species-specific reference spectra. Because the protein composition of most bacterial species is uniform and stable, the matching of sample to reference spectra allows for unambiguous identification.
The MALDI-TOF technology is currently being used only to identify pure bacteria or fungal isolates; nevertheless, new research is showing promise for the direct application of this testing technology to clinical samples which may have mixed microbial populations, such as CSF, urine, or blood. Full validation of these procedures in the near future will allow us to determine bacterial presence within 2-3 hours of a sample arriving at the laboratory to help direct treatment in critical cases.

The deployment of the MALDI-TOF in the Athens Veterinary Diagnostic Laboratory in 2015 has allowed us to substantially reduce the turn-around for microbial identification, accompanied by antibiotic susceptibility determination (MICs) without increasing costs.

Although there are several brands of MALDI on the market, we chose the BioMerieux Vitek MS because of its superb compatibility features which allow seamless interfacing with Vitek 2 sensitivity equipment to provide minimum inhibitory concentration data in a shorter time.

MALDI-TOF — A mouthful of a name for sure, and more physics than you may care to know, but certainly a great innovation to produce the accurate and timely results that help veterinarians provide the best care possible to both companion and production animals.
Dr. Guilherme (Gui) Verocai
New Director of the Parasitology Diagnostic Laboratory

Dr. Guilherme (Gui) Verocai is the new Director of the Parasitology Diagnostic Laboratory in Athens. He has a DVM and Master's degree in Parasitology from Brazil, a PhD from The University of Calgary, Canada, and did a postdoctoral fellowship at The University of South Florida. He is currently an Assistant Research Scientist in the CVM Department of Infectious Diseases. In addition to his service at the Diagnostic Lab, Dr. Verocai is involved in teaching Parasitology and the Clinical Parasitology Rotation for veterinary students. His areas of research interests are diagnostic parasitology, filarial parasites of veterinary and medical importance (e.g., canine heartworm), and the biodiversity of parasitic worms of wild and domestic animals. In his spare time Dr. Verocai likes to go hiking with friends, traveling, and tending to his freshwater planted aquarium.