Welcome to the fall 2015 issue of our newsletter, Diagnostic Veterinary Matters. We would like to thank all of our clients and stakeholders for your steadfast support and loyalty over the years, which recently culminated in legislative approval for the second $1.5 million equipment bond issue implemented in the Georgia FY16 annual budget. Your continuous support for the third and last installment of a $650,000 equipment bond request in FY17 will allow us to complete our replacement of equipment and to purchase new technology for improved diagnostic testing, decreased turn-around time and enhanced disease surveillance in the state of Georgia.

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MESSAGE FROM THE DIRECTORS

We want to welcome Dr. Rebecca Wilkes, our newly hired Microbiologist/Virologist and Molecular Biologist at the Tifton Laboratory. Dr. Wilkes came to us from the University of Tennessee, School of Veterinary Medicine (UTSVM) where she obtained her DVM and PhD degrees in 2001 and 2007 respectively. Since 2008 she has performed both research and diagnostic testing on multiple viral animal diseases at the UTSVM. Please see her biography on page 2 of this newsletter. She brings to our Laboratory System a wealth of knowledge and experience in Microbiology, Virology and Molecular Biology that will greatly enhance our ability to continuously meet your current and future needs. Her expertise in Molecular Biology and Bioinformatics will allow us to move into the next generation of diagnostic testing involving Next Generation Sequencing and Whole Genome Sequencing.

In August, our outreach team hosted our annual CE event. This year’s theme was “S is for Skin” which focused on a variety of small animal dermatological conditions. We were privileged to be the first to hold an event in the beautiful Alumni Hall at the new Veterinary Medical Center. Thank you to everyone who joined us for an impressive turn out. We are already looking forward to next year. In addition, our team hosted a booth at the fall Georgia Farm Bureau Commodity Conference and the Georgia Cattlemen’s Association Athens-Oconee “Round-Up”. Our next event will be the fall GVMA conference in November. Please stop by our booth to meet the outreach team and register for some exciting prizes.

Please, do not hesitate to contact our outreach team to let us know how we can better serve your specific needs or the general needs of the state for continuous improvement of animal health and human well-being.

Murray E. Hines II
Jeremiah [Jerry] T. Saliki

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www.vet.uga.edu/dlab
Mycoplasma bovis is an important component of the bovine respiratory disease complex (BRD), often as a secondary opportunist, but can act as a primary pathogen as well. Indeed, in South GA M. bovis is commonly isolated from calves and young adult cattle succumbing to bronchopneumonia. Throughout the Spring and Summer of 2015, the TVDIL has confirmed several cases of Mycoplasma pneumonia thus far. This is not a new finding at the laboratory; a retrospective study showed that between 1999 and 2013, 62.1% of TVDIL bovine bronchopneumonia cases were positive for M. bovis via immunohistochemistry (Turnquist S and Rajeev S, 2014). Along with respiratory disease, this bacteria can also cause polyarthritis, otitis, mastitis, and conjunctivitis.

The bacteria lacks a cell wall and will adhere to the respiratory epithelium following exposure, commonly from contaminated milk or from nasal secretions of an infected individual. The organism is adept at evading the host immune response and consequently it can be difficult for infected animals to clear the infection. In addition, due to the lack of a cell wall, M. bovis is resistant to a variety of antibiotics (e.g. Ampicillin, Ceftriaxone, oxytetracycline) and animals may not respond well to therapy. Additionally, the bacteria is a primary opportunistic pathogen and can cause secondary infections such as pneumonia, meningitis, and pododermatitis.

Clinically, affected animals are typically febrile, dyspneic, have a persistent cough, and often have mucopurulent discharge. Animals with concurrent lameness and/or swollen joints would increase suspicions of M. bovis infection. On postmortem examination, M. bovis infections result in a necrotic-suppurative bronchopneumonia with variable diameter, nodular areas of abscession. Suppurative exudates are often apparent in the trachea and nasal cavity as well. Multiple joints should be evaluated for evidence of arthritis/tenosynovitis (i.e. distended with fluid and fibrin deposits). Histologically, in affected lung sections, the airways (bronchioles and bronchi) are often distended with neutrophils, necrotic cellular debris, and macrophages. The nodular lesions in the lung correspond to large foci of caseous necrosis encased by fibrous tissue and infiltrates of macrophages. Necrotic foci will frequently undergo dystrophic calcification.

These gross and histologic changes in a calf/cow with bronchopneumonia are suggestive of M. bovis, however, additional diagnostics would be required to confirm such an infection. Bacterial culture specific to Mycoplasma can be performed on affected fresh tissue or swabs of the lesions. In the event of a positive culture, PCR is used to confirm or possibly exclude M. bovis. In addition, immunohistochemistry for M. bovis is available at our laboratory and can be performed on formalin-fixed paraffin embedded tissue. This test is highly specific for M. bovis infection. The TVDIL often performs Mycoplasma cultures on milk samples. Serology for detection of M. bovis exposure is available. These are useful ante-mortem tests for screening groups of animals.

Mycoplasma pneumonia in cattle is often complicated with concurrent/underlying bacteria or viral infections. Bacteria often identified in conjunction with M. bovis include: Mannheimia hemolytica, Pasteurella multocida, and Histophilus somni. Viral agents often involved are: Bovine Viral Diarrhea virus, Bovine Herpesvirus – 1, Bovine Respiratory Syncytial Virus, and Bovine Parainfluenza virus – 3. The Tifton laboratory routinely tests for these agents in cattle with respiratory disease.

**NATURAL HISTORY**

In North America, canine flu is predominantly an H3N8 Type A influenza. This influenza jumped species from horses to dogs around 1999 or earlier, transferring the entire genome as a unit. It was recognized, and subsequently isolated, in a series of outbreaks that occurred in racing Greyhounds in the Southeast US in 2004. Banked blood samples from Greyhounds that had been at tracks with respiratory disease outbreaks between 1999 and 2003 were later documented to exhibit seroreactivity to the H3N8 strain. Prior to this, no canine-adapted version of the influenza A of the dog. There has been no evidence of dog-to-human transmission of H3N8, nor was there evidence of horse-to-human transmission of this strain of influenza A prior to the existence of the canine-adapted version.

Curiously, H3N2 influenza appears to have jumped into the canine species on the Korean peninsula around the same time, likely through recombination events with various avian influenza viruses known to circulate in that area. In 2015 an outbreak of canine flu in and around Chicago was documented to be the first cluster of H3N2 cases in North American dogs. A third canine influenza strain, H5N2, isolated in China can be spread from dog-to-dog, but sustained spread through pet populations has not yet been described. Influenza viruses are prone to recombination in co-infected hosts (termed “antigenic shift”), and this tendency is likely to continue to impact the emerging diversity of canine influenza strains.

Since influenza is a novel virus in the canine species, susceptibility is high. This is because the population has no native or historical immunity. This immunological naivety is in contrast to species such as humans, birds, and pigs, which have a long and well-documented history of influenza A infection. In such species a background to moderate level of immunity persists, based on prior exposure to related strains of the same agent. Antigenic shift and drift by the virus impact the efficacy of that historical immunity, and occasional epidemics result when the antigenic change is substantial.

Lacking background immunity, dogs as a species should be expected to experience an epidemic of flu, and indeed the disease has been spreading slowly through population clusters since 2004. However, not all highly contagious or highly prevalent diseases are lethal. The ongoing canine flu epidemic in North America is proving to exhibit lower mortality than had been initially feared.

Like other respiratory pathogens, canine influenza A is spread by respiratory secretions, respiratory aerosols, and fomites. The enveloped RNA virus is not highly durable in the environment, and can be readily killed by common disinfectants, including quaternary amonium compounds and bleach. At this point in time, the infectivity rate seems to be at least 80%. To reiterate, this means that healthy adult dogs exposed to the virus will contract the infection in 80% of cases, because they have no acquired or natural immunity to any similar agent unless they have been deliberately vaccinated. The incubation period prior to the onset of clinical signs is 2-4 days, however, dogs are shedding contagious virus during this incubation period.

Once infected, the morbidity rate is approximately 75%. That is to say, of that 80% of dogs that actually become infected, ⅔ will develop clinical signs. Conversely, up to 25% of naturally-infected dogs do not ever exhibit any clinical signs, although they may still be shedding the virus. Clinical signs begin to manifest while viral shedding by the infected dog is tapering. The typical clinical signs are fever, coughing, sneezing and malaise, which are indistinguishable from more classic causes of canine infectious tracheobronchitis (i.e., “kennel cough” or “cough”) in an individual dog. Things that might raise suspicion for canine flu include rapid spread through a population of otherwise healthy and/or vaccinated adults, and potentially higher rates of fever. In most cases, the disease will be self-limiting, or responsive to supportive care, and dogs will recover within 2-3 weeks. As with all canine influenza, complicating pneumonia is the most dangerous sequelae, the very young, or the previously unhealthy are at greatest risk of complications.

Early data suggested mortality rates were up to 10%. However, those estimates included the relatively small number of track greyhounds who died early in the discovery of disease, and mortality estimates have continued to be revised downward as the disease spreads through the pet population. At the time of writing, most estimates mortality rate at 1-5%. While the recent Chicago outbreak garnered a lot of media attention, there is no evidence that the H3N2 strain is more virulent. In H1N1, regular press reports are accurate, over 1500 dogs were diagnosed with the disease and 6 deaths were reported, which would equal a mortality rate of 0.4%.

**DIAGNOSIS**

The disease is indistinguishable from other canine influenza, is self-limiting in most cases, and has no definitive therapy. However, achieving a definitive diagnosis may still be of value, particularly in a shelter, kennel, or clinic outbreak. The UGA Athens Veterinary Diagnostic Laboratory (uvagvetlab.org) offers a PCR test panel for canine flu and other agents of canine influenza, including *Bordetella bronchiseptica*, Canine adenovirus 2 and 3, Canine distemper, and Canine coronavirus. The sample of choice for this panel is nasal or pharyngeal swabs in a red top tube with a few drops of sterile saline, shipped prior over night with ice packs.

PCR is a definitive test in rule out test for dogs showing acute clinical signs such as fever. However, false negative PCR results may occur after about one week of clinical disease. In such cases serologic testing (available through several commercial laboratories) is useful, provided two serum samples taken 2-3 weeks apart (first sample should be collected within a week of onset) are tested and a fourfold rise in titer is observed. Early in the course of the epidemic, a single positive titer was considered diagnostic because there was no background seropositivity in dogs. With the advent of a vaccine, and a growing population of dogs that have recovered from natural infection, a single positive titer is no longer considered sufficient to confirm the diagnosis.

**THERAPY**

Therapy is supportive as for all respiratory viruses, and complicating bacterial pneumonia will require the most aggressive care. Dogs should be allowed - encouraged to rest, and kept well-hydrated, clean and warm. Some clinicians use prophylactic broad-spectrum antibiotic therapy in anticipation of secondary bacterial infection, particularly in very young, very old, or otherwise immunocompromised dogs. Racing Greyhounds in the early outbreak phases experienced higher mortality; it is unclear whether this was due to virus specific or husbandry-related factors. Aggressive supportive care including broad-spectrum antibiotic therapy may therefore be indicated in infected Greyhounds.

Oseltamivir has been used by some clinicians to treat this and other viral diseases of dogs. The author does not employ this agent for several reasons. In most instances of canine influenza, specific antiviral therapy would not be required because the disease is self-limiting. Limited safety or efficacy studies of oseltamivir in dogs exist, and no studies of oseltamivir in dogs infected with influenza exist. Finally, given the ongoing concern about pandemic human influenza, it seems prudent to minimize risk of induction of resistance by avoiding use of antiviral agents whenever possible.

**PREVENTION**

A killed virus vaccinated (Novivac® Canine Flu H3N8, Merck Animal Health), given as a two-dose initial series with an annual booster, was approved by the USDA for conditional release in May 2009 and granted full release in June 2010.

Experience would suggest that influenza vaccines with a modified-live agent might be an efficacious way to induce protective local immunity that excludes the virus from the site of infection. However, concerns of recombination, which may be relevant to human influenza epidemiology, the USDA will not currently consider licensure of any MLV influenza products for canine use. Limited studies have documented the efficacy of the killed H3N8 vaccine, and have largely been performed in laboratory settings. Overall, vaccinated dogs seroconverted after initial doses, demonstrated rising titers after booster doses, and exhibited minimum four-dilution anamnestic responses after challenge. Coughing was the predominant clinical sign in vaccinated and control dogs alike, and was more prevalent, subjective, more severe, and of longer duration in the unvaccinated controls. Results were similar whether the challenge strain was the homologous H3N8 vaccine strain, various field H3N8 strains, or co-challenged with canine parainfluenza. The efficacy of the killed H3N8 vaccine against experimental or natural challenge with H3N2 virus.

Currently, H3N8 influenza vaccine is considered a non-core vaccine for dogs, and is recommended for a similar population of dogs that receive *Bordetella bronchiseptica* vaccines, that is, dogs that travel, board, show, or are regularly exposed to novel other dogs. While some clinicians have not chosen to vaccinate in regions of the country without a documented outbreak, it takes only a single infected dog to bring the virus into a new area, and most dogs are asymptomatic sheds for a period of time during the infection. Thus, the risk factors for *Bordetella* infection and canine influenza infection are the same in any region. In regions of the country where outbreaks have occurred, some kennels, dog parks and other canine facilities have begun to require the vaccine for dogs entering their populations.

Again, influenza viruses are prone to recombination (antigenic shift) and there is no research into H3N8 vaccine efficacy against novel or recombined strains such as H3N2. Influenza viruses are also prone to antigenic drift away from the original type, and recent (2011) findings confirmed that canine influenza virus does exhibit ongoing mutation during single infections. Ongoing investigation into vaccine efficacy against novel and emerging strains is warranted.

For more information, clients can be referred to the following AVMA site.

https://www.avma.org/public/PetCare/Pages/CanineInfluenza.aspx

Practitioners can follow emerging developments with the AVMA at

https://www.avma.org/KB/Resources/Reference/Pages/Canine-Influenza-Backgrander.aspx

Testing is available at the UGA Athens and Tifton diagnostic laboratories (http://uvagvetlab.org), other state veterinary laboratories and some private laboratories.

To contact the UGA Veterinary Diagnostic Laboratories, call 706-542-5588 or 229-386-3340.

**References**


**Canine Flu: Status Report and Vaccine Update**

By Dr. Kate E. Creevy, DVM, MS, DACVIM

College of Veterinary Medicine, UGA, Athens GA
Antimicrobial pharmacodynamics – Relationship to therapeutic success

By Brent Credille, DVM, PhD, DACVIM
Food Animal Health and Management Program, CVM, UGA

Antimicrobial agents are the most widely used class of pharmaceuticals in veterinary medicine. In choosing the appropriate antimicrobial agent, the practitioner must consider: (1) the likely identity of the infecting microorganism(s), (2) their typical in vitro susceptibility patterns or the clinical response in patients infected with the same pathogens, (3) the nature and site of the infectious disease process, (4) the drug formulation and dosage regimen required to maintain appropriate concentrations at the site of infection, (5) the safety of the drug, (6) the withdrawal time of the drug at the dosage used, and (7) the cost of therapy. The goal of this manuscript is to review antimicrobial pharmacodynamics, antimicrobial susceptibility testing, and how integration of this knowledge can help improve therapeutic success. For any disease caused or mediated by an infectious agent, the efficacy of antimicrobial therapy is dependent upon three factors:

1. Susceptibility of the pathogen to the chosen antimicrobial.
2. Characteristics of drug exposure necessary for optimum response.
3. Concentrations of free drug at the site of infection.

This relationship, the interaction of systemic drug exposure and corresponding clinical effects, is termed the pharmacokinetic/pharmacodynamic relationship (PK/PD). Here, pharmacokinetics is best defined as the handling of the drug by the host (i.e. what the body does to the drug) while pharmacodynamics is defined as the effect of drug on microorganisms over time (i.e. what the drug does to the bug). It is the pharmacokinetic relationship between a specific antimicrobial and disease-causing microorganism that is the focus of this discussion. Optimal dosing of antimicrobial agents is dependent on both the pharmacokinetic and pharmacodynamics properties of a drug. Currently, the most widely utilized pharmacokinetic input is plasma drug concentration and the minimum inhibitory concentration (MIC) the primary pharmacodynamic input.

Bacterial antimicrobial susceptibility is determined in vitro using one of several available tests. Disk diffusion, concentration-gradient agar dilution, and broth dilution (macro or micro) have all been used to evaluate susceptibility to antimicrobials. Disk diffusion provides mostly qualitative information (susceptible, intermediate, resistant) while both the broth dilution and concentration-gradient agar dilution tests provide quantitative data (MIC). With these tests, the MIC is defined as the lowest concentration of antimicrobial that inhibits the growth of target bacteria. It is important to note that inhibition of bacterial growth rather than bacterial killing is the primary endpoint. The designation of a microorganism as susceptible or resistant is determined by comparing the organism’s MIC to breakpoints established by the Clinical Laboratory Standards Institute (CLSI). Breakpoints, defined as the concentration of drug above and below which specific bacterial isolates are characterized as either susceptible, intermediate, or resistant, are determined by 3 criteria:

1. Range of in vitro MICs of an antimicrobial for a representative population of specific bacterial pathogens.
2. PK/PD parameters established on the basis of the relationship between drug concentrations and microbial susceptibility.
3. Results of clinical trials in the target species.

When these in vitro susceptibility tests are presented to the clinician or researcher, a pathogen will be designated as susceptible, intermediate, or resistant, designations defined as:

- **Susceptible**: An infection caused by the specific isolate may be successfully treated with the recommended dosing regimen of an antimicrobial agent approved for that disease process and inhibiting microorganism.
- **Intermediate**: An infection by the isolate can be treated at body sites where drugs are physiologically concentrated or when a high dosage can be used. This designation also represents a “buffer zone” that should prevent minor technical factors from causing major discrepancies in interpretations.
- **Resistant**: An infecting isolate is not inhibited by typically achievable concentrations of a specific drug with a standard dosing regimen.

It is important to note that clinical breakpoints are only relevant for specific bacteria, a specific drug, and a specific organ system. Thus, breakpoints established for ceftiofur against Mannheimia haemolytica in the respiratory tract are irrelevant when that organism is the cause of disease within another body system (mammary gland, uterus). Generally, when species-specific breakpoints are not available for a disease condition, breakpoints are adapted from humans or other domestic animal species. Examples of this approach include penicillin G, tetracyclines, potentiated sulfa, amnoglycosides, and protein syntheses.

For these antimicrobial agents, a susceptible result is certainly better than a resistant one, however, there are no data available to correlate the results of susceptibility testing and expected outcome in veterinary species and the data obtained from these susceptibility tests must be interpreted with caution. In these situations, knowledge of an infecting organism’s MIC combined with pharmacokinetic data ideally describing the concentration of drug within the tissue of interest can assist in predicting efficacy.

The ability of in vitro susceptibility testing to predict outcome depends on whether the susceptible and resistant breakpoints have been correlated to clinical efficacy in the species of interest. Very few breakpoints have been established specifically for veterinary medicine. The Clinical Laboratory Standards Institute (CLSI) has approved bovine respiratory disease specific breakpoints:

- Penicillin
- Flomfenicol
- Spectinomycin
- Tetracycline
- Tildipirosin
- Tilmicosin
- Tulathromycin

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Breakpoints attempt to take an in vitro test result and extrapolate it to an in vivo response; however, disease outcome is influenced by factors such as host immune status, variations in individual pharmacokinetic parameters, or increased disease severity/prolonged disease duration.
The Receiving Section at the TVDIL includes two full-time employees. The duties of this section include receiving and sorting through all samples arriving at the laboratory and entering the information from each submission into our computer LIMS system. Johnne Graves has been employed at the TVDIL for 18 years. She graduated from Tift County High School in 1968 and attended Medical College of Georgia where she received a 2-year degree in Radiology Technology. She and her husband have farmed for 44 years and she spends most of her extra time outside on the farm.

Mary Ann Ethridge has been employed at the laboratory since 2003. She graduated from Tift County High School in 1984 and she attended Abraham Baldwin Agricultural College for 2 years. Her primary hobby is dancing with her youngest daughter who has special needs. Her daughter is on a dancing competition team which travels around Georgia and Florida where her daughter performs solo dances and has won top honors with her performances. Mary Ann also has another daughter who attends Abraham Baldwin Agricultural College, works part-time and assists taking care of her little sister.

The Athens Veterinary Diagnostic Laboratory is pleased to welcome our newest team members. Jillian Fishburn joined our Serology and Virology department as a Laboratory Manager I. She comes to us with her Master’s from the University of Georgia and experience working as a Research Professional in other laboratories here on campus. The Bacteriology and Molecular departments welcomed two new Laboratory Technicians, Juan Sanchez and Sierra Farris. Both Sierra and Juan are graduates of the Biotechnology program at Athens Technical College. Our newest addition to the Accessions team, Lara Jackson, also has her Master’s from UGA and has worked in another laboratory within the university system. Each one of these individuals shares our enthusiasm for diagnostic medicine and is eager to provide our clients with the highest level of customer service.