INFECTIOUS DISEASE TESTING 2012: A PRACTICAL APPROACH

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INTRODUCTION

Gone are the days when testing for infectious diseases was simple. There was culture for most bacteria, serology for heartworm, many of the rickettsia and fungal organisms, cytology, fecal float and smears and…really not much more. Well that has changed over the last few years:

- There are many more organisms to worry about and their prevalence is so much higher than previously known
- There is more and more evidence that diseases previously not thought of as infectious do at least have an infectious component
- Because of the high prevalence and in some cases evidence of chronic infection - proving disease in many cases is very different than proving exposure or even infection
- Co-infection has emerged as a tremendously important phenomenon making a diagnosis of a single infection often not sufficient for a diagnosis or to explain the clinical disease course
- New testing modalities such as traditional and real-time PCR are different that anything we have known before, are complicated to understand and interpret sometimes and are offered by many different labs

What then are the options for the veterinary practitioner?

- Give up on a diagnosis and just treat everything with doxycycline
- Try to master latest literature and figure out ideal test for each organisms and each clinical syndrome
- Use combination and triggered testing based on grouping organisms by body system and clinical syndromes.

What are the requirements then for this combination trigger based testing?

- Multi modality approach
- Offered to practitioners based on region, life style and clinical syndrome of the pet
- Differentiate exposure from active disease
- Differentiate disease from vaccination
Offered in a reasonable platform regarding sample collection, cost and turn – around time, and communications between the lab and the practitioner regarding optimal testing

This lecture will concentrate on examples of this approach combining in-house serology, reference lab quantitative serology, real-time PCR and culture for different disease states and clinical syndromes.

LYME DISEASE AND TICK BORNE DISEASE TESTING:

Canine Lyme and the causative agent *Borrelia burgdorferi* have become a huge problem in many areas of the United States and Europe over the last 20 years. Over the last 5 years the disease has spread considerably and the incidence has risen considerably, even in areas of the Northeast that previously only had sporadic cases. The dilemmas facing practitioners regarding this disease are many. Regarding clinical Lyme cases the first dilemma is how to diagnose the disease. How to verify that a dog that is sick, and serologically positive – actually has Lyme disease. What diagnostics are appropriate in a case like this? The second, and often easier decision the practitioner must make is regarding treatment – with what? How much? and for how long. Often the harder question is regarding non-clinical dogs. When and then how to monitor non-clinical dogs for evidence of *Borrelia burgdorferi* infection? And, most importantly, how to prevent Lyme disease? How good is tick control? Should we or should we not vaccinate? Who should we vaccinate and do vaccines prevent or promote the most severe manifestation of Lyme disease – Lyme nephritis? This talk will briefly review the most updated information regarding the practical aspects of the diagnosis and treatment of canine Lyme disease. It will then focus on prevention of clinical Lyme disease. Should we monitor non-clinical dogs for *Borrelia burgdorferi* infection? And if we do - what do we do with a positive result? Does treatment prevent clinical disease? Should all dogs be treated or are there ways to pick and choose? And then should we vaccinate? What vaccines are there? Do the vaccines work? How do they work? Are there risks to vaccinating Lyme negative dogs? Lyme positive dogs? Using new data this talk will revolve around the ways that veterinarians can answer those questions best in their practice.

LYME DISEASE: CLINICAL SIGNS

The lack or apparent lack of clinical signs in most dogs with active Lyme infection makes both the diagnosis and the study of this disease very difficult. In dogs, clinical signs are observed only in approximately 10% of infected cases. These signs tend to occur 2 – 5 months after the infection and include lameness – mono or polyarthritis, lymphadenopathy, lethargy, and fever. Skin lesions are uncommon in dogs. These signs typically resolve within approximately 3 days, in some case only with antibiotic therapy. Some questions remain regarding more serious, less common syndromes that have been associated with Lyme infection in dogs including: Renal disease (Lyme nephritis), Cardiac disease (myocarditis) and neurological disease. Another question yet to be answered is whether some dogs get the devastating chronic recurrent disease as seen in some infected humans.
**DIAGNOSIS**

**What diagnostic tools are available?**

1. **Bacterial culture or PCR.** This is very difficult in the case of Borrelia due to the small number of infecting organisms and the complicated techniques involved in their successful culturing. Blood PCR testing is not recommended as a screening test for this organism as many infected dogs will have a negative PCR result.

2. **Serology.** Clinically today we must rely on serology in conjunction with clinical signs to diagnose this disease. There are currently 3 types of serological testing commercially available:
   a. **Non-specific ELISA.** This is a very sensitive test aimed at identifying any antibodies produced against Borrelia whole cell antigen. It does NOT differentiate between antibodies produced in reaction to Lyme infection vs. Lyme vaccination and will be positive in both instances. Since we can usually never be sure of infection status and many times of vaccination status a positive non-specific ELISA should ideally be followed up with an additional test that would conform infection like a Western blot or a C₆ antibody test.
   b. **C₆ antibody testing.** There are currently two commercially available tests for canine antibodies against the Lyme C₆ protein. This protein is expressed only during infection, therefore these tests are meant to be positive only in the event of natural exposure and negative in naive dogs or dogs vaccinated for Lyme. These tests include the in-house 3Dx or 4Dx SNAP tests and the quantitative C₆ antibody test available through IDEXX. Logical use of these tests:
      i. The SNAP 4Dx is an excellent test for screening dogs for Lyme infection. This can be done as part of a screening program for asymptomatic dogs or when Lyme disease is suspected. A positive result is indicative of active Lyme infection.
      ii. The quantitative C₆ antibody test tests for similar antibodies as the 4Dx but in a quantitative fashion. The value of knowing the quantitative C₆ titer is still unproven at this time, but we have shown that the titer correlated very well with circulating anti-Lyme immune complexes and is likely a very useful tool in treatment decisions of non-clinical dogs. The practical questions of when and how to use this tool will be addressed using new data. Briefly, it appears that the pre-treatment serum quantitative C₆ antibody concentration does predict immune complex load and can be used to assess value of therapy or possibly risk of vaccination. A follow up C₆ antibody concentration does suggest successful treatment in humans and possibly in dogs as well.
   c. **Western blot.** This technique involves a blot smeared with Lyme antigens located in known locations. This is a relatively expensive and labor-intensive type of test and its interpretation requires expertise. Therefore this will never be in-house technology. As we have learned more about the 4Dx and quantitative C₆ assays, they have taken some of the role of the Western blot as a confirmatory assay. At this time I would recommend using the Western blot only in dogs where the vaccinal status is important to the veterinarian.
d. Other tests –
   i. Cornell University Animal Health Diagnostic Laboratory – To my knowledge there is no published validation of this test for dogs. Its utility is unknown at this time in my opinion.
   ii. Abaxis – There is talk of a new Abaxis test, it has not been validated and is not yet available to my knowledge and so is also of unknown utility.

The use of a tick borne disease panel combining 4Dx, quantitative serology and real-time PCR for a variety of tick borne diseases will be discussed including a case presentation.

**CANINE LEPTOSPIROSIS**

Leptospirosis is an important worldwide zoonosis, caused by an infection with a pathogenic species of the genus *Leptospira*. These are highly motile obligate aerobic spirochetes that share features of both Gram-negative and Gram-positive bacteria. Dark field or phase contrast microscopy is necessary to visualize these bacteria since they stain so poorly. This genus is classified today based on genetic determinations. Most of the commonly diagnosed canine pathogenic serovars are still classified (as before) as belonging to the *L. interrogans* species although the serovars *grippotyphosa* and *ballum* are now classified as belonging to the *L. borgpetersenii* and the species *L. kirschneri* respectively. This talk will include a general overview of the current literature regarding canine leptospirosis. This will include the most recent thoughts on the epidemiology of the disease, the pathogenesis, the methods of testing for the disease, prevention and vaccination as well as clinical signs and treatment.

**PATHOGENESIS**

Understanding the molecular basis for Leptospiral virulence is crucial in the effort to produce more effective vaccines. Identifying surface antigens that are expressed during active infection in vivo may also facilitate distinction between active infection and vaccination or exposure. For example, *Leptospira* immunoglobulin-like protein A (LigA) contains domains homologous to proteins with attachment and invasion functions and is expressed in vivo but not in vitro. *Leptospira* organisms penetrate abraded skin or mucus membranes and replicate rapidly in the bloodstream. The sequence of events after infection likely depends on:

1. Virulence. Important questions include: Is there a difference between serovars? New data that will be presented would suggest that there is.
2. Immune response. Questions: Has the dog been vaccinated or previously exposed? How well does the vaccine protect from natural infection and is there acquired immunity after being infected with a specific serovar? Many of the commercially available vaccines have been shown to provide good short term immunity but the length of that immunity is unknown. A recent study comparing different commercially available vaccines should only a mild serological response to a series of 2 vaccinations but good immunity when challenged 1 month after the second vaccine.
3. Gender – Some studies show males as being more likely to be clinically affected than females.
After infection the following organs may be affected:

1. **Kidneys**: Renal colonization. Organisms persist and multiply in the renal tubular epithelial cells causing acute nephritis. If not fatal and not treated appropriately - this MAY lead (info is mostly experimental from *L. canicola*) to chronic interstitial nephritis and a persistent carrier state.

2. **Liver**: Liver damage. Centrilobular necrosis and sub cellular damage, bile canaliculi and duct occlusion may cause icterus. This is not seen as commonly today as with icterus.

3. **Blood vessels**: Vasculitis and DIC due to endothelial damage – likely more common that we think.

4. **Lungs**: Pulmonary hemorrhage. This is common in severe cases in people. The incidence of canine cases is unknown.

5. **Uterus**: Abortion? Unknown significance in canine patients.

6. **Eyes**: Uveitis? Common in horses but can occur in dogs as well.

7. **Brain**: Meningitis and encephalitis have been documented in severe cases in humans. The incidence of canine cases is unknown.

8. **Immune system**: Secondary immune mediated disease (poly-arthritis, hemolytic anemia etc...). The incidence of canine cases is unknown.

**DIAGNOSIS**

The diagnosis of canine leptospirosis is not definitive in most cases in veterinary practice today. It is based on a combination of appropriate clinical signs, clinico-pathological and imaging data as well as serology. The definitive diagnosis would require identifying the organism in urine or tissue, which is very uncommon because of the technical difficulties of culture and direct visualization of the organism and the high sensitivity of the organism to antibiotics (a dose or two of antibiotics can cause a negative culture, urine FA and even a negative urine PCR!).

**Clinical signs**: The clinical signs may depend on the serovar, the virulence, the immune status of the patient and the organ targeted by the bacteria. Although most cases diagnosed today in dogs are associated with acute renal failure other syndromes exist as well. Published data will be presented describing the frequency of clinical signs and clinico-pathological features documented in a large study recently completed by the author at Cornell University.

**Serology**: The microscopic agglutination test (MAT) is the most commonly used test in veterinary medicine. The highest dilution of serum that agglutinates 50% of the *Leptospira* organism is the titer. This test does have a high specificity and sensitivity, especially if it is repeated 2-3 weeks after a negative result when leptospirosis is suspected. How specific is this test for serovars? There is a large degree of cross reactivity. We assume that the serovar with the highest titer is the one causing the infection. Disappointingly, this was found to be true only in about 50% of the cases in a human study comparing MAT titers to cultures. In veterinary medicine a titer to a non-vaccinational serovar of 1:800 or greater or a fourfold rise in titer is commonly thought to be suggestive of active disease. Vaccinational titers are typically low but have been documented up to 1:3200. Antibiotic therapy may cause a decrease in the MAT titer or prevent a rising titer when convalescent titers are performed. An ELISA test is the most commonly used test for screening in humans. It is not serovar specific and if used in dogs it should probably be confirmed by MAT. ELISA testing can be performed for IgM and IgG. The IgM ELISA may be positive prior to the MAT titer in an acute infection. Direct fluorescent antibodies (FA) can be useful on tissue and occasionally urine. The organisms though tend to disappear within a day or two of therapy causing a high number of false negative results.
Newer testing that is not yet widely commercially available in veterinary medicine includes Western blot analysis and PCR. Western blots may be used in the future to differentiate titers originating from vaccinations from natural exposure. This is extremely important now that common pathological serovars have been included in the canine vaccine. We have shown that although there are many common bands in Western blots of dogs that were infected and dogs that were vaccinated the pattern is different. Natural infection causes many more bands in a more complex pattern. This technique will likely be used in the future. PCR identification of Leptospiral DNA in urine, blood, CSF aqueous humor and tissue has been used in human medicine and veterinary research and has been recently made available on a commercial basis by IDEXX Laboratories®. Unfortunately we have found that even one or two days of antibiotics may cause a negative urine PCR. Thus obtaining samples prior to antibiotic use may be a key to the use of PCR as a screening test. We now recommend this test if pre-antibiotic samples can be obtained from a dog with appropriate clinical signs, especially if early in the disease process when the MAT is likely negative. A positive PCR at a laboratory with a well validated test is good evidence for infection. A negative PCR never rules out infection. A flow chart incorporating PCR with MAT will be discussed. Recent vaccination does NOT interfere with real time-PCR testing.

Cases including additional clinical syndromes utilizing additional diagnostic combination testing will be presented.