Testing for Tick Borne Diseases: How and When?

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Tick borne diseases (TBDs) used to be quite common in Greyhounds, and some believe they are still very prevalent. A fair number of people involved in Greyhound rescue consider it more cost-effective to "treat" dogs for TBDs than to test them and treat only the positive ones. This approach should be frowned upon, since:

a. not all TBDs respond to doxycycline or imidocarb
b. adverse reactions to imidocarb are common (and sometimes severe in Greyhounds)

In our experience with Greyhounds over the past 17 years, the prevalence of TBDs has decreased markedly, likely due to better tick control. For example, 15 years ago approximately half of the retired racing Greyhounds evaluated as potential blood donors at OSU tested positive for *Babesia canis* or *Ehrlichia canis* serology, whereas now only 1-3% of the dogs are positive for any TBD. However, in some diagnostic laboratories, the prevalence of positive dogs using serology is still high, even though the results are frequently not reproducible when the same sample is analyzed by a different lab or method ("the never-ending conundrum...").

Interpretation of test results may differ in healthy versus sick patients, and depending on whether serology or PCR are used (examples will be used during the lecture). Dogs with symptomatic TBDs usually have one or more of the following: weight loss, fever, anemia, thrombocytopenia, neutropenia, lymphadenopathy, ocular signs, splenomegaly, and polyarthritis.

**Canine Monocytic Ehrlichiosis**

**Serologic Diagnosis**

It is uncommon to diagnosis canine monocytic ehrlichiosis by recognition of morulae in circulating mononuclear cells. The diagnosis is generally suspected based on clinical and routine laboratory findings and confirmed using serology. The serological assays most widely used are the indirect fluorescent antibody (IFA) test, which is available at most commercial laboratories, and the Snap 3Dx (IDEXX Laboratories, Inc., Maine, USA) for clinic use. IFA is not species-specific so animals infected with *E.canis, E. chaffeensis* and possibly other ehrlichial and anaplasma species may test positive due to cross-reactivity. In addition, caution should be exercised in diagnosing CME in animals that have low titers, or animals that have titers but clinical findings not really consistent with CME. Interpretation of the results, even with experienced laboratory personnel is subject to reagents used, state and quality of microscope, and observer experience. Current recommendations are that a positive diagnosis is made if you have a single titer of >1:64 with consistent clinical / laboratory findings, or changing titers on paired, acute and convalescent serum samples.

The SNAP 3DX (IDEXX Corporation, MN) is an in-house method for serological testing of animals suspected of having monocytic ehrlichiosis. The Snap 3Dx assay, which uses recombinant analogs of the major outer membrane proteins (p30 and p30−1), has increased specificity and sensitivity over the older Snap Canine Combo from IDEXX, which used whole-cells from the *E. canis* Oklahoma strain as antigens. Testing and comparison of this assay with known IFA-positive and negative samples indicates a very high specificity (near 100%), but slightly lower sensitivity (79.2%), which means that a positive result implies a very strong probability of infection with either *E. canis* or *E. chaffeensis*, but 20% of the IFA-positive animals tested were negative by the SNAP test. These animals most often had IFA-titers of
<1:300. So if you are using this assay in a low-prevalence area, you have an excellent positive and negative predictive value, but if testing a high prevalence population, such as limiting testing to animals with clinical and laboratory finding consistent with CME the negative predictive value is lower due to higher potential for false negatives. Animals infected with either E. canis or E. chaffeensis will test positive using this or any of the currently available serological assays. The only way to distinguish between these two infectious agents is by in vitro cultivation or PCR analysis (available at the North Carolina State Univ. Tick-Borne Disease Testing Laboratory).

Identification of Organisms

Although serology is probably the most sensitive way to diagnose monocytic ehrlichiosis, it is also the least specific. By nature, most serological assays detect circulating antibodies to a particular infectious agent which could mean active infection, previous exposure, previous vaccination (if available) or cross-reactive antibodies to a different organism altogether. Because of this, clinical and/or clinicopathologic findings must concur with a positive titer in order to confirm active infection. Alternatively, and more specifically, the organism itself may be identified in the patient, thus confirming an infectious state regardless of clinical findings. Unfortunately, in most cases of the monocytic forms of canine ehrlichiosis, organisms are not typically observed in circulating mononuclear cells. This is a very uncommon finding and microscopic identification of the organism is not a useful method of diagnosis. The more sensitive methods of identification are culture and the polymerase chain reaction (PCR).

E. canis and E. chaffeensis can be cultivated in vitro, but this process requires tissue cell culture. Therefore, this technique is primarily used for research or investigative purposes and not for clinical diagnoses. However, PCR analysis is routinely conducted by many research and commercial laboratories. This method of analysis requires a sample of whole blood in anticoagulant (EDTA). By extracting the DNA from the patient’s blood, and using specific "primers" that will identify and amplify organism DNA, the PCR assay can detect very minute amounts of organism in an infected animal’s blood. Levels or circulating agents that can be detected by PCR analysis are far below what would be detected by microscopic evaluation alone.

Because of the similarity in clinical findings and cross-reactivity associated with the various infectious agents, PCR analysis is the most reliable way to distinguish between species within the genera Ehrlichia and Anaplasma. However, due to the low numbers of circulating organisms cases of monocytic ehrlichiosis, false negative results may occur with PCR analysis. This is most commonly seen in chronically infected carriers or in animals where blood samples are drawn for analysis after the institution of antimicrobial therapy resulting in parasitemia that is below the level of sensitivity of the assay. False positive results are typically due to accidental contamination of the sample from micro amounts of aerosol DNA in the lab.

Canine Granulocytic Ehrlichia
(Ehrlichia ewingii and Anaplasma phagocytophilum)

Serologic Diagnosis

Since the organism cannot be cultured in vitro, there are currently no serological assays for the diagnosis of infection with E. ewingii. Some animals infected with E. ewingii may test positive on IFA tests for E. canis, but this cross-reactivity is inconsistent. IFA tests for the diagnosis of canine A. phagocytophilum infection are available at commercial laboratories, however, the assay may be listed under the old nomenclature, IFA for E. equi. When requesting this assay, the laboratories must be aware of the species of the patient (canine or equine). The same problems of false positive results may occur with this assay as described above for monocytic ehrlichiosis. Some animals infected with A. phagocytophilum may test positive on IFA tests for A. platys (see below), but this cross-reactivity is inconsistent. Animals infected with A. phagocytophilum will not test positive on assays designed to diagnose E. canis infections (SNAP 3Dx).

Identification of Organisms
Unlike with monocytic ehrlichiosis, in the granulocytic forms of these diseases, organisms can usually be found in low numbers of circulating neutrophils in the peripheral blood and/or synovial fluid from infected animals. However, these two species cannot be distinguished from each other by clinical findings or routine laboratory diagnostics. As mentioned above in monocytic ehrlichiosis, PCR analysis is required for species identification. The assays are similar to those described above.

**Anaplasma platys**

*Anaplasma platys* (formerly *Ehrlichia platys*) causes infectious cyclic thrombocytopenia in dogs. This agent is unique in that it is the only intracellular infectious agent described in man or animals to specifically infect platelets.

**Serologic Diagnosis**

This organism is not able to be cultured in vitro, however, an IFA test for antibodies against *A. platys* has been developed (available at the diagnostic laboratory at Louisiana State University, School of Veterinary Medicine). Although data is not yet available, there is likely cross-reactivity when testing animals infected with *A. phagocytophilum*.

**Identification of Organisms**

Diagnosis of clinical infection with *A. platys* can be made by observing organisms within platelets. PCR analysis is also available for this agent (see monocytic ehrlichioses).

**Canine Babesiosis**

**Diagnosis**

A definitive diagnosis of *B. canis* infection is made by identification of the organisms in stained-blood films. Serological diagnosis can be made using IFA tests, but some cross-reactivity occurs between babesial species. High titers suggest current infection, but IFA tests may be negative in acutely infected animals, especially pups. If parasites are not recognized in the blood, it is difficult to differentiate babesiosis from autoimmune hemolytic anemia, because both disorders may be Coombs' test positive.

**Babesia gibsonii**

**Diagnosis**

The clinical diagnosis of infection with *B. gibsonii* is often made by microscopic examination of a peripheral blood film. The small (1-2.5 µm) round to oval piroplasms are usually identifiable in erythrocytes of clinically infected animals. The organisms are detectable as early as 1 week post-infection.
and peak parasitemias are seen by 3 to 4 weeks. Peak parasitemias in most dogs range from 2% to 6% of erythrocytes infected, however, parasite numbers may in some cases be much higher. Many infected animals will be Coombs’ test positive. Therefore, the diagnosis must be distinguished from IMHA. Specific species identification is accomplished by PCR analysis of infected, whole blood. PCR analysis is currently available through the North Carolina State University Tick-Borne Disease Testing Laboratory, or through the Department of Pathobiology at Auburn University. The parasitemia in subclinical carriers (especially seen in pit bull and Staffordshire terriers) is too low to be detected microscopically and PCR analysis must be done to confirm a carrier state.

Serology using the IFA test demonstrates the presence of antibodies in serum directed against these organisms, but some cross-reactivity occurs between babesial species. Titers > 1:80 are considered significant, but most infected animals have > 1:320. Both false negatives and false positives can occur using the IFA test. Speciation can only be done using PCR analysis.