Supplementary Information 7: Vector-borne Disease Screening – General Principles

Diagnostic testing

The use of PCR and serological tests in screening for vector-borne disease has recently been reviewed in an ACVIM Consensus Statement.[1] Briefly, PCR testing for most vector-borne diseases is highly sensitive. However, some organisms, including *Babesia*, *Bartonella*, *Ehrlichia*, *Rickettsia* and *Anaplasma* species can circulate in peripheral blood intermittently and in low numbers.[2-9] Therefore, even though the diagnostic sensitivity of PCR may be very high, the clinical sensitivity in an individual patient can be low. Similarly, the sensitivity of serological testing can be low in the acute phase of infection, prior to seroconversion. For some organisms such as *Bartonella* and possibly *Babesia spp*, seroconversion may not occur in some patients.[10-12] Cross-reactivity between species and genera limit specificity of serological testing. Serology also does not distinguish between previous exposure and active infection. Combining PCR with serological testing enhances sensitivity.[6,13,14] Repeat testing, including repeating PCR on the same or additional samples, and pairwise serological testing to demonstrate a four-fold change between acute and convalescent titers, is also necessary to document infection in many cases.[6-9,13,14]

Risk of exposure and infection with vector-borne disease

Although the distribution of vector-borne disease tends to follow its vectors, it is important to consider that the geographic distribution of many tick vectors and their associated pathogens is rapidly expanding.[15-18] Co-infection should be ruled out, especially if one agent is found during screening. For example, *Rhipicephalus sanguineus* is the confirmed or suspected

vector for multiple organisms, including *B. vogeli*, *E. canis*, *A. platys*, hemotropic *Mycoplasma spp*, *B. gibsoni*, and *Bartonella spp*.[2,17,19-21] Therefore, in the United States, *B. vogeli* should be ruled out in a dog with IMHA seropositive to *E. canis* on initial screening.

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