Ehrlichiosis Information

Tests available at VBDDL: Serology (IFA and IDEXX Snap4DXplus) and PCR

Six known *Ehrlichia* spp.; *E. canis (Rhipicephalus sanguineous), E. chaffeensis (Amblyomma americanum), E. ewingii (A.americanum), Panola Mountain Ehrlichia (A. maculatum), E.rumminantium (A.variegatum) and E.muris*-like agent *(Ixodes scapularis)* are capable of infecting domestic animals and, at times, people with worldwide distribution of the associated genera of tick vectors. As several species have not been cultivated in cell lines, availability of species specific IFA can be limited. A degree of cross reaction between species can partially overcome this limitation and the development of synthetic peptides is increasing the test options. PCR, with properly designed primers, can identify genus and species specific DNA in blood samples of actively infected animals.
Link to 2002 ACVIM consensus statement on Ehrlichiosis: Ehrlichia

Disease (Animals with the following are reasonable candidates for evaluation for ehrlichiosis.)

- History of tick attachment.
- fever, myalgia, lethargy
- reluctance to move, polyarthritis
- bleeding diatheses, hemorrhage /circulatory collapse
- Thrombocytopenia

Testing

- Examination of blood smear. Not offered at VBDDL since <5% of cases show morulae upon examination. *Ehrlichial* spp. morulae are morphologically similar but infect different host blood cell types: *E.canis*, *E.chaffeensis*, and *E.muris* infect monocytes while *E.ewingii* and *E.ruminantium* infect granulocytes.
- Serology: (requires 2mls serum): VBDDL utilizes a combination of immunofluorescent antibody (**IFA)** with *E.canis* antigen (antibody titers ≥1:64 indicative of prior or current infection with *E.canis, E.chaffeensis* and possibly *E.ewingii*) and an ELISA based kit (IDEXX Snap® 4DXPlus) that detects *E.canis, E.chaffeensis* or *E.ewingii* but does not distinguish between the species.
- Polymerase chain reaction (**PCR**): Amplification of a specific piece of DNA from the organisms of interest. Since Ehrlichia live in white blood cells, EDTA whole blood (**2mls**) is the sample of choice. Obtain samples BEFORE treatment, since treatment may reduce numbers of organisms and result in false negative test results. VBDDL tests on a genus level for all known *Ehrlichia* spp. utilizing 16S rDNA, *sodB* and/or *groEL* genes. The presence of *Ehrlichia* DNA in the blood indicates active infection. Samples positive at the genus level are further tested to identify species. Animals with extensive tick exposure can be infected with more than one *Ehrlichia* species.

Treatment

For ehrlichiosis, the cellular and humoral antibody response can be ineffective resulting in chronic infection. **Doxycycline** (5-10mg/kg every 12 hours for 4 weeks for dogs) is the drug of choice with therapeutic dosage varying according to host species. Doxycycline treatment in dogs of only 2 weeks has been shown to be inadequate for *E. canis* infections. Short term prognosis following treatment for canine ehrlichiosis is generally very good with clinical improvement usually occurring within 24-48 hours in dogs with acute phase or mild chronic-phase disease.

Periods up to a year may be necessary for complete hematological recovery. The long term prognosis following treatment is much more variable, potentially related to failure to diagnose concurrent infections. Undiagnosed infection with a *Babesia* or *Bartonella* spp. can be misinterpreted as an ineffective therapeutic response when treating ehrlichiosis, as doxycycline is generally an ineffective treatment for babesiosis and bartonellosis. Experimentally, enrofloxacin will suppress the clinical manifestations of *E. canis* infection and may result in hematological improvement, but does not eliminate the infection. Although imidocarb dipropionate has gained clinical acceptance in some endemic regions for treating severe or refractory cases of ehrlichiosis, lack of efficacy has been demonstrated in both naturally and experimentally infected dogs. Dogs co-infected with other tick borne pathogens, may respond over a longer period of weeks or relapse following doxycycline treatment. Animals are not resistant to re-infection following successful clearance of bacteria, therefore the use of safe and effective acaracide products are critical to help prevent re-infection.

Ehrlichia canis - Canine monocytic ehrlichiosis (CME) spread by *Rhipicephalus sanguineus* ticks is a chronic infection and even following therapy (Doxycycline 5-10mg/kg every 12 hours for 21 days for dogs), antibodies may persist for prolonged periods of time (6 months or longer). The acute and chronic stages of CME can be detected clinically; dogs appear ill and develop clinical, hematological and biochemical abnormalities. The subclinical stage of CME, lasting months to years, is not associated with clinical signs of illness and therefore may go unnoticed by pet owners and undiagnosed by veterinarians unless antibodies are detected during annual screening with in-clinical kits. *E.canis* or *E.canis*-like infections in humans and cats have been rarely detected.

Ehrlichia chaffeensis - Susceptibility to infection with *E. chaffeensis* has been confirmed in dogs, coyotes, red foxes, white-tailed deer and humans [human monocytic ehrlichiosis (HME)]. *Odocoileus virginianus,* the white-tailed deer and larval or nymphal stage *Amblyomma americanum* ticks are considered the principal reservoir and vector of *E.chaffeensis.* In dogs, clinical signs of illness are generally mild to moderate in severity and include fever, anorexia, lethargy, thrombocytopenia, non-regenerative anemia, leucopenia and elevated ALT activity.

Ehrlichia ewingii - As is true for *E.chaffeensis*, white-tailed deer are the primary reservoir for *E.ewingii, A.americanum,* the Lone Star tick, the primary vector and susceptibility to infection has been confirmed in dogs and humans. This tick is spreading with the expansion and movement of deer populations from southern and central regions of the US north and eastward all the way up into Canada. *E.ewingii* resides in host neutrophils. Dogs can be chronically infected with *E. ewingii,* but most clinically relevant infections are considered acute. Clinical signs in dogs consist of transient fever, anemia, thrombocytopenia, leukopenia and polyarthritis, most often with few accompanying biochemical abnormalities. Dogs experimentally infected with *E. ewingii* develop disease signs approximately 10-14 days after inoculation, antibodies after 3-5 weeks of infection and remain seropositive for up to a year. Previously infected dogs are not immune to reinfection although disease symptoms may be milder or resolve faster. Co-infections with *E.chaffeensis* and *E.ewingii* detected serologically and by PCR are second only in prevalence to co-infections with *Borrelia burgdorferi* (Lyme Disease) and *Anaplasma phagocytophilum* from the *Ixodes scapularis* deer tick.

Ehrlichia muris – A 2011 report from the upper Midwest USA, identified *E.muris,* or a closely related *E.muris*-like (EML) agent, in humans, most of whom were immunocompromised (Pritt et al, 2011). Recent data supports the emergence of this new *Ehrlichia* species, potentially

transmitted by *I. scapularis*, in dogs as well (Hegarty et al 2012). Human patients presented with fever, headache, thrombocytopenia, lymphopenia, and elevated levels of liver enzymes (Pritt et al, 2011). Until specific serological assays have been developed, the only methods to definitively identify infection with this novel *Ehrlichia* species would be cell culture isolation or PCR amplification and DNA sequencing. The PCR methods of the VBDDL will detect this pathogen.

Ehrlichia ruminantium and **Panola Mountain *Ehrlichia* -** *E.ruminantium*, the causative agent of Heartwater is primarily a disease of ruminants (cattle, sheep and goats) causing great economic impact in South Africa and the Caribbean transmitted by a variety of *Amblyomma* spp ticks in all their developmental stages. This *Ehrlichia* sp. invades plasma cells, neutrophils and the endothelial cells of the capillaries. Molecular evidence has demonstrated a strain in clinically ill dogs in South Africa. There are concerns relative to the possible spread of Heartwater into the USA via the natural or commercial importation of infected birds and animals that might allow transmission to *Amblyomma* spp in North America (*A.maculatum*, *A.cajennense*, or *A.americanum*). A strain unique to the US, in fact, was found in dogs, goats, deer, and humans transmitted by *A.maculatum* in the southeastern US named Panola Mountain Ehrlichia. Symptoms of Heartwater, after an incubation period of 3-10 days, include intermittent high fever, respiratory distress, diarrhea, inappetance, lethargy and moist cough. Treatment in ruminants with tetracyclines (10-20mg/kg in one or 2 IM doses) is effective in the acute phase but, if not diagnosed before the onset of CNS symptoms, prognosis in the chronic phase is poor. Morbidity depends upon strain virulence. Different strains do not induce homologous or heterologous cross protection.

Insights gained from VBDDL associated research.

- In the summer of 1997, the VBDDL undertook an unfunded investigation of a kennel of sick and dying Walker Hounds routinely used for deer hunting in rural North Carolina. A high degree of co-infection was documented by serology and molecular assays. By PCR, of the 27 dogs, 15 were infected with *Ehrlichia canis*, 9 with *E.chaffeensis*, 8 with *E.ewingii*, 3 with *Anaplasma phagocytophilum*, 9 with *A.platys*, 20 with a *Rickettsia* species, 16 with a *Bartonella* species, and 7 with *Babesia canis*. Both *E. canis* and an uncharacterized *Rickettsia* species appeared to result in chronic or recurrent infection. As an outcome of this study, *Babesia* primers to detect *B.canis* and *B.gibsoni* were developed and lessons were learned in the detection of co-infecting species with an appreciation for high risk populations exposed to numerous ticks. Published article: Kordick SK, Breitschwerdt EB, Hegarty BC, Southwick KL, Colitz CM, Hancock SI, Bradley JM, Rumbough R, McPherson and MacCormack JN. Coinfection with Multiple Tick-Borne Pathogens in a Walker Hound Kennel in North Carolina JClinMicrobiol. 1999; 37:2631–2638.

- A treatment study in *E.canis*-infected dogs showed:
  - Doxycycline hyclate (5mg/kg every 12 hours for 14 consecutive days) eliminated infection in 8/8 dogs with acute *E. canis* infections.
  - Prior infection did not infer protection against challenge with homologous (strain Florida) or heterologous (strain NCSU Jake) strains of *E. canis*.

  Published article: Breitschwerdt EB, Hegarty BC, Hancock SI. Doxycycline Hyclate treatment of experimental canine Ehrlichiosis followed by challenge inoculation with two *Ehrlichia canis* strains. Antimicrobial Agents and Chemotherapy, 1998; 42:362–368.

- Several field trials and studies have been conducted in collaboration with IDEXX Labs (Westbrook, ME) to assist in R&D of point of care screening devices.

- Field trials using thousands of serum samples obtained by our diagnostic service have tested the sensitivity and specificity of the SNAP®3DX, 4DX and 4DXPlus formats.
- To clarify the clinical relevance of annual *E. canis* screening with IDEXX kits, blood from *Ehrlichia canis* SNAP 3Dx positive dogs was tested by PCR for the presence of *Ehrlichia* spp. Thrombocytopenia was frequently detected in healthy *Ehrlichia* SNAP 3Dx-positive dogs, whereas active infection was infrequently confirmed by PCR (14%, 12/86). We concluded that treatment based upon such screening kits alone is not always warranted, supporting the manufacturer's recommendations that positive test results be followed by complete blood counts and PCR.

Published articles: (1) O'Connor TP, Hanscom JL, Hegarty BC, Groat RG, Breitschwerdt EB. Comparison of an indirect immunofluorescence assay, western blot analysis, and a commercially available ELISA for detection of *Ehrlichia canis* antibodies in canine sera. Am J Vet Res. 2006;67:206-10.
(2) Hegarty BC, Diniz PPVP, Bradley JN, Lorentzen L, Breitschwerdt EB. Clinical Relevance of Annual Screening Using a Commercial Enzyme-Linked Immunosorbent Assay (SNAP 3Dx) for Canine Ehrlichiosis. J.Am Anim Hosp Assoc. 2009; 45: 118-124.

Individual cases that have been referred to and tested using the array of serological and molecular methods offered by the VBDDL have resulted in the detection of novel *Ehrlichia* spp in cats, dogs, and horses.

- *E. canis*-like infection in 3 cats with clinical manifestations consistent with canine ehrlichiosis but lacking antibodies to *E. canis* antigens was detected by PCR. The predominant disease manifestation was polyarthritis in 1 cat and bone marrow hypoplasia or dysplasia accompanied by pancytopenia or anemia and thrombocytopenia, in the other cats. *16S* ribosomal DNA, amplified from EDTA blood samples from each cat, was identical to each other and was identical to a canine isolate of *E. canis*. To date, no further reports of *E.canis*-like infections have been found in cats. The research arm of the VBDDL is currently testing feline serum samples for exposure to *Ehrlichia/Anaplasma/Borrelia* to detect unrecognized Vector Borne Diseases in cats.
  Published article: Breitschwerdt EB, Abrams-Ogg AC, Lappin MR, Bienzle D, Hancock SI, Cowan SM, Clooten JK, Hegarty BC, Hawkins EC. Molecular evidence supporting *Ehrlichia canis*-like infection in cats. J Vet Intern Med. 2002;16:642-9.

- *E.muris* was confirmed by PCR in a dog from Minnesota in early September in which *Anaplasma* species but not *Ehrlichia* antibodies were detected using SNAP 4Dx. IFA against *E.canis* antigen was also negative, but PCR using *16S* primers that detect both *Anaplasma* and *Ehrlichia* spp yielded results that when sequenced matched *Ehrlichia muris*. A *GroEL* PCR amplicon also showed 100% homology with *E. muris*. Published article: Hegarty BC, Maggi RG, Koskinen P, Beall MJ, Eberts M, Chandrashekar R, and Breitschwerdt EB. Ehrlichia muris infection in a dog from Minnesota. J Vet Intern Med 2012;26:1217–1220.

- Panola Mountain *Ehrlichia* (PME) in a dog from NC that was seroreactive to *Ehrlichia canis* (1:1028) antigens by IFA, but seronegative to *Ehrlichia* spp. peptides in the SNAP 4Dx Plus kit. Because of the discrepancy, *Ehrlichia* spp. PCR was performed that upon sequencing determined an infection with PME. In this report, we provided molecular evidence of PME in a thrombocytopenic dog with abnormal lymphocytosis and clonal T-cell expansion. Treatment with doxycycline resulted in resolution of thrombocytopenia, abnormal lymphocytosis and abnormal lymphoid cells in liver and lymph nodes, supporting a potential role for PME as a cause of host immune dysregulation. Published article: Qurollo BA, Davenport AC, Sherbert BM, Grindem CB, Birkenheuer AJ, Breitschwerdt EB. Infection with Panola Mountain Ehrlichia sp. in a dog with atypical lymphocytes and clonal T-Cell expansion. J Vet Intern Med. 2013.

- Collaboration with veterinarians at Oregon State University has detected a novel Ehrlichia species in horses in Nicaragua. "*Ehrlichia* species DNA was amplified from four *Ehrlichia* spp. seroreactive horses from Mérida, Nicaragua. Sequencing of 16S rDNA, *sodB* and *groEL* genes indicates that the bacterium is most likely a novel *Ehrlichia* sp. The tick vector, and the potential for canine and human infections, is currently unknown." Published article: O'Nion VL, Montilla HJ, Qurollo BA, Maggi RG, Hegarty BC, Tornquist SJ, Breitschwerdt EB. A Potentially Novel *Ehrlichia* Species Identified in Nicaraguan Horses. EID 2014 in press.